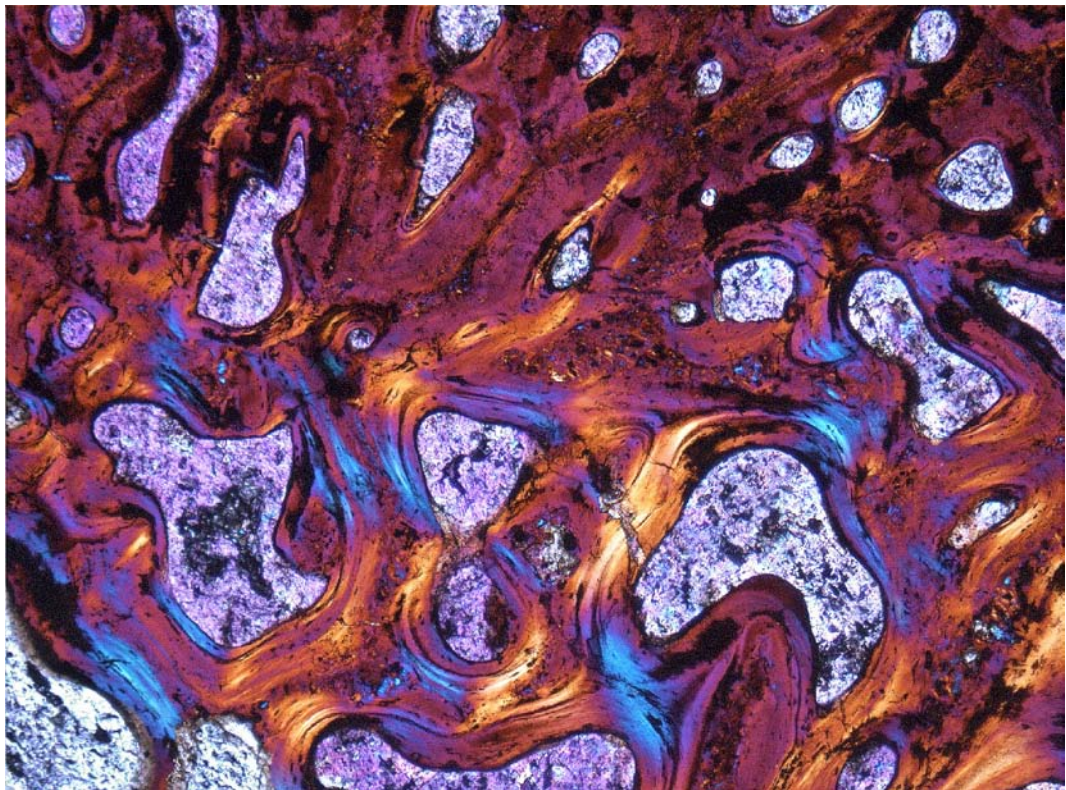


Master Thesis, Department of Geosciences

Gross internal structure and microstructure of plesiosaur limb bones from the Late Jurassic of Svalbard

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FACULTY OF MATHEMATICS AND NATURAL SCIENCES

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Master Thesis in Geosciences

Discipline: Palaeontology

Department of Geosciences and Natural History Museum, Oslo

Faculty of Mathematics and Natural Sciences

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Thank you!

The Natural History Museum in Oslo is my favourite museum. Thank you for hosting the exhibition with the animated dinosaurs in 1993, making a seven year old girl interested in palaeontology, and for giving me the possibility to continue this seven years later when I was here for a week during a school project. That is how it began. Since 2009 I have been a part of the Spitsbergen Jurassic Research Group, with field work at Svalbard in August 2009 and 2010 and writing of this thesis at the museum with Jørn Hurum as my supervisor.

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Lene
Oslo, May 2011

Abstract

In this study plesiosaur limb bones of four specimens from the Late Jurassic of Svalbard have been studied to map gross internal structure and microstructure and compare this to extant marine reptiles and mammals. Two specimens, one juvenile (*species A*) and one subadult (*species B*) are from the Adventdalen Group, Janusfjellet subgroup, Agardhfjellet formation, Slottsmøya member, dated as mid-Volgian; one adult (*species D*) from Agardhfjellet, also dated mid-Volgian; and one juvenile (*species C*) of unknown age. The bones examined are propodials, phalanges, mesopodials and metapodials. This study is the first to describe the microstructure of the latter two. The inner bone structure fits an active marine animal living in cold water. Many of the features in the present material are often found in animals with rapid growth and high metabolism, including secondary osteons, high vascularization, pits on the outside of the epiphysis, woven and possibly fibro-lamellar bone. The long bones have two endochondral cones in a periosteal sheath, with a small medullary cavity. The propodials have a defined and quite compact cortex in the subadult, a finding that rejects the view that all plesiosaur bones became more porous through ontogeny. There is a microstructural difference between bones from different ontogenetic stages: juvenile bones have no remodeling and not completely ossified endochondral cones. Three bones have regular trabecular rings in a porous cortex, maybe resulting from cyclic growth caused by seasonality, migration or ontogeny.

Introduction

Plesiosaurs

Plesiosaurs (Sauropterygia, Plesiosauria) were secondarily aquatic marine reptiles, up to 15 metres in length and one of the main marine animal groups globally distributed in the Mesozoic. The first plesiosaurs originated in the Late Triassic, had a large diversification during the Jurassic and the Cretaceous, and became extinct in the Late Cretaceous (Brown 1981; Mazin 2001). About 43 genera and 65 species are recognized (Mazin 2001), but there are major gaps both geographically and temporally in the plesiosaur fossil record (Druckenmiller 2006). Two main groups of plesiosaurs are recognized: the long-necked plesiosaurs with small heads (Plesiosauroidea) and the short-necked pliosaurs with larger heads (Pliosauroidae) (Brown 1981). Both types are found in the Spitsbergen deposits. Sauropterygia has an uncertain position within the Reptilia, and the origin and ancestry of sauropterygians, and which sauropterygians are the closest relatives to the plesiosaurs, is not known (Klein 2010).

Plesiosaurs had an euryapsid skull, meaning only a single temporal fenestra dorsal to the postorbital and the squamosal. Fossils suggest this is a condition derived from a diapsid ancestor (Liem et al. 2001). The plesiosaur limb was very derived and important for propulsion, with fore- and hindlimbs of almost the same size and shape (Liem et al. 2001; but see discussion in Munthe-Kaas 2011). The propodials were large, while other limb bone elements were reduced in size and became more similar in shape through evolution. Hyperphalangy elongated the distal part of the limb. The limb is thought to have been a stiff unit, but there is a debate about how it was used and how the animal propelled itself through water (Thewissen & Taylor 2007; Munthe-Kaas 2011).

Plesiosaurs were carnivorous (Druckenmiller & Russell 2008) and maybe endothermic (Bernard et al. 2010). Their reproductive mode is not known, but their body shape seems incompatible with laying eggs on land, even though some plesiosaur fossils have been found close to the shore. This will remain unsolved until a plesiosaur that is pregnant or giving birth, or a plesiosaur egg, is found (Mazin 2001). Pregnant early sauropterygians are claimed to have been found, but this is uncertain (Cheng et al. 2004), as is the description of a plesiosaur embryo (Moodie 1911).

Histology

Fossil bones are subjected to many factors and both shape, size, mineral composition and colour are often changed with time. But the integrity of the histology is often preserved and can be studied in thin sections. This provides a considerable amount of information and is used for studies of animals from the earliest to extant vertebrates (Chinsamy 1997). Common structures to describe and use for interpretation are the degree and system of vascularization, porosity, fibrillar organization and bone tissue type, relationship between cortex and medulla, presence and orientation of osteocyte lacunae and canaliculi and degree of remodeling.

Four principal factors determine the type and form of hard tissues in vertebrates: phylogeny, ontogeny, mechanics and the environment (de Ricqlès 1977; Horner et al. 2000), but it is unclear to what degree each of these factors results in the observable structures. There has been a debate about whether bone microstructure mainly reflects phylogeny or ontogenetic and functional factors (including growth rate and biomechanical constraints), but it seems that “microstructural characters may have both a functional significance and a systematic value at some level of the phylogeny” (Cubo et al. 2005: p.562). Between the observable characters and the factors that influence it, there are several interrelationships (Montes et al. 2010). Caution should therefore be taken in interpretation. Using microstructural characters for inferring phylogeny has been applied to sauropterygians (Klein 2010) and correlation between microstructure and ecology has been used for mosasaurs (Sheldon 1997) and plesiosaurs (Wiffen et al. 1995).

Plesiosaur bone microstructure

The first article on bone microstructure of plesiosaurs described a vertebra and was published in 1878 (Hasse 1878). Around year 1900 the strange inner structure of the plesiosaur limb was discussed: it looked like an hourglass broken in the middle, or two large cones, which are also known to field palaeontologists, because the cones are sometimes freed from the surrounding sheath or have collapsed (Fig. 1). Kiprijanoff (1883a, 1883b) first produced illustrations of the cones and did thin section studies on the plesiosaur limb (Figs. 2A, B, C). Lydekker (1889; Fig. 2D) called the cones a “remarkable peculiarity” and thought they were epiphyses, the latter a mistake corrected by Moodie (1908), who also proposed a theory on how the plesiosaur limb grew (1916; Figs. 2F-I). Other early work on the structure and growth of plesiosaurian propodials was done by Williston (1903, Fig. 2E) and Woodward (1898, as referred in Moodie 1916). After this, hundreds of papers have been written about plesiosaurs

(e.g. see references in Druckenmiller & Russell 2008), but only a few are concerned with the inside of the bone, and no one has described the growth process. It has been suggested that the inner structure results from ossification of cartilaginous cones, “but this is uncertain and ground sections need to be investigated” (Haines 1938; 1969: p. 92). This study, together with other similar studies, will answer that question.

In the early 20th century, survey studies of bone microstructure in different animal groups included plesiosaur limb bones (Seitz 1907; Gross 1934; Nopcsa & Heidsieck 1934). More recently, a few studies have been done on the microstructure of the plesiosaur limb bones (propodials and phalanges) (Wiffen et al. 1995; Fostowicz-Frelik & Gazdzicki 2001; Salgado et al. 2007; Gren 2010), focusing mainly on ballasting or lightening of bones as an adaptive mechanism, and the degree of internal remodeling. Most of this work is on Late Cretaceous species. Other plesiosaurian bones that have been studied through their microstructure are ribs, vertebrae, girdle bones and gastralia (Enlow & Brown 1957; Cruickshank et al. 1996; Street & O’Keefe 2010). These studies were also mainly focusing on ballasting, while plesiosaur bones also have been thin sectioned to study diagenesis and preservation (Martill 1991; Pewklian et al. 2008).

Other sauropterygians for which bone microstructure has been studied are *Placodus* (de Buffrénil & Mazin 1992), *Pachypleurosauria* (de Buffrénil & Mazin 1989; Sander 1990; Klein 2010), *Nothosaurus*, *Cymatosaurus* and *Pistosaurus* (Krahl et al. 2009; Klein 2010). In addition the marine reptiles ichthyosaurs (de Buffrénil & Mazin 1990) and mosasaurs (Sheldon 1997) have been studied in this way.

Fossil material cannot be tested experimentally. It is necessary with comparisons to extant animals to uncover the links between growth rate, other factors and bone microstructure (Starck & Chinsamy 2002). Finding the best taxa for comparison to plesiosaurs is not easy, since the closest extant and extinct relatives of Sauropterygia are not known. Extant marine reptiles can be used, for instance marine turtles (Rhodin 1985) and crocodiles (Lee 2004; Klein et al. 2009). But there is no real extant marine counterpart to the plesiosaurs. Both ecologically and possibly physiologically they resemble more extant marine mammals than reptiles.

There are striking similarities in bone structure in animals secondarily adapted to aquatic life. Many well-documented examples of convergent evolution are known, both for the gross anatomy and the inner organization of the bone structure (Fig. 3). Two main patterns exist, basically very heavy and very light bones. Animals that live close to the shore and that

do not depend on swimming fast often show a local or general increase in skeletal mass, by processes called pachyostosis, osteosclerosis or pachyosteosclerosis. This is known for several groups of amphibians, reptiles, birds and mammals, and the process is often connected to a heterochronic development where calcified cartilage persists and remodeling is inhibited. Very light bones occur in animals that live in the open sea and use rapid and sustained swimming. Their bones are very porous, because compact bone is replaced by cancellous tissue in a process called osteoporosis. This specialization is found in many cetaceans (Fig. 3C, D), crocodiles, turtles, frogs and ichthyosaurs (Hua & de Buffrénil 1996; summary in de Ricqlès & de Buffrénil 2001). Wiffen et al. (1995) concluded that plesiosaurs change bone porosity during life, from a ballasting adaptation in juveniles to very porous bones in adults, and this has been widely cited.

Bone growth and body growth

Bones appear in the embryo as a condensation of mesenchyme, which chondrifies into a cartilage template of the bone (Liem et al. 2001). The cartilage grows in size and is gradually replaced by bone. In the diaphysis the cartilage is first calcified, followed by vascular invasion and replacement by bone. Bone consists of two phases, organic collagen which makes a framework onto which the inorganic phase, hydroxyapatite crystals, are bound (Chinsamy-Turan 2005). The whole process is called ossification, and the patterns and sequence of ossification in different bones have been investigated in different animals and can give valuable information (e.g. neonatal squamates in Maisano 2001; plesiosaurs, ichthyosaurs and mosasaurs in Caldwell 2002).

Being hard, bone cannot grow by inner expansion, only by deposition of new bone to so-called appositional surfaces: outside and inside of the bone, inside canals and in cancellous bone (Enlow 1963). Bone is deposited on the outside from a tissue called the periosteum (derived from the embryonic perichondrium), making periosteal bone, and on inner surfaces from the endosteum. Endochondral bone is endosteal bone that replaces cartilage (Reid 1996). Two processes act at the same time during the life span of a bone: deposition and resorption, the latter being a process adapted to hard bone tissue that maintain the shape of the bone and remodel it by removing bone in some areas while bone is deposited in others (Enlow 1963). The literature is conflicting about the level of internal remodeling in plesiosaur bone. This information may be important in order to understand how much the plesiosaur limbs changed during ontogeny and to compare them to extant reptiles.

In most limb bones, the periosteal tissue is a sheath that constrains the shape of the bone and thus makes the classic dumb-bell shape of long bones (Farnum 2007), while the endochondral forms the inner matrix and the articular faces at the ends, beneath a cartilage cap (Caldwell 1997a). There are however large differences as to how the two components work together. In many animals, growth in length is accomplished by endochondral bone growth beneath the epiphyseal cartilage, with resorption from the periosteal side happening at the same time (Enlow 1969), while in other, the periosteal also plays a role in bone elongation (Farnum 2007). In non-long bones, endochondral bone is the main component, and through evolution, some long bones, for instance in plesiosaurs, completely lose the periosteal component (Caldwell 2002).

Body growth is obviously one of the most important processes for an animal to accomplish, and skeletal growth is necessary for overall growth. There is a very large variation in growth rates, at which stage an animal is born, and the postnatal growth strategy (Farnum 2007). Growth rate and the presence or absence of determinate growth together result in the growth strategy (Farnum 2007). These factors are not yet investigated for plesiosaurs, but the use of histological research might give answers. Histology has been used to infer growth patterns for several extinct animals (e.g. for *Pterodactylus* in Chinsamy et al. 2009). The classic “Amprinos rule” says that differences in bone tissue types are reflections of variation in bone depositional rates. According to this slow growth gives lamellar bone matrix, while rapid bone formation results in fibro-lamellar bone (Chinsamy-Turan 2005). This has been tested for different animals (e.g. Starck & Chinsamy 2002) and in a phylogenetic context by Montes et al. (2010). These experiments show that no simple connection exists, even though the rule can be said to be true qualitatively (Chinsamy-Turan 2005).

Determinate growth is growth that ends at a given time and cannot resume, while indeterminate growth is when growth has the potential to continue for the entire life span of the animal. In both cases, rapid growth is common at first followed by a plateau (Farnum 2007). Reasons for reaching a specific size can be mechanical constraints, need to be a certain size to eat certain prey, or an energetic limit to growth (Sebens 1987). Indeterminate growth has also been defined as cases where size is correlated with age, but this definition includes invertebrates, which have more flexible growth patterns (Sebens 1987). In this work the first definition is used.

Many animals that have determinate growth have a secondary ossification center in the epiphysis, while also other structures have developed (Enlow 1969). Indeterminate growth in bones can occur if the epiphysis remains cartilaginous or if a secondary ossification center is present but no bony union occurs between epiphysis and diaphysis. In addition to secondary centers of ossification lamellated bone or peripheral rest lines in tissue deposited late in ontogeny might indicate determinate growth (Chinsamy 1997). Animals lacking a secondary ossification center cannot necessarily be assumed to have indeterminate growth, but in such cases addition of bone is usually possible (Farnum 2007).

Determinate growth is common in mammals and birds, but exceptions include elephants and rodents (Sebens 1987). Indeterminate growth is by far the most common for fish, non-avian reptiles and amphibians, but signs indicating determinate growth have been found in pteranodons (Bennett 1993), dinosaurs (Curry 1999; Sander 2000) and among extant reptiles such as varanids (de Buffrénil et al. 2005). There has been no study devoted to find out whether plesiosaurs had determinate growth.

Another feature of bone growth is whether it is constant or proceeds in cycles, the latter often based on seasonal interruption, most commonly alternating cold-hot or wet-dry seasons. Cycles of sexual activity and migration might also cause cyclic growth. Such changes in growth can be recorded in the bones, for vertebrates in dermal bones of the head region, vertebral centra and limb bones (Peabody 1961). When cyclic growth gives structural results in the bone, it is often as relatively uniform bands, termed zones or “growth rings”, in the periosteal cortex. This is common in reptiles (Enlow 1969) and such bone is called zonal bone. They zones are bounded by rest lines (often termed lines of arrested growth) if the growth stops and starts, or annuli if they result from periods of faster and slower growth (Reid 1996; Chinsamy-Turan 2005). Zonal patterns are seen mainly in ectotherms, while continuous growth is known mainly from endotherms, but exceptions do occur (Reid 1996). Zonation is reported for plesiosaurs in most summaries on their microstructure (de Ricqlès 1976; Wiffen et al. 1995). In extant reptiles a single zone is usually formed per year (Chinsamy-Turan 2005). “Growth rings” have been used in many cases to establish the age of a single individual (e.g. Bryuzgin 1939), but this has also been contested, since rings can have different origins, and the earliest formed rings might disappear during remodeling of the bone.

Geological setting

The plesiosaur fossils in this study from the 2009 field season (PMO 216.839 and 216.838) were found in the Late Jurassic deposits from Sassenfjorden area of central Spitsbergen, Svalbard (Fig. 4). They were found in the Adventdalen group, Janusfjellet subgroup, Agardhfjellet formation, Slottsmøya member (Dypvik et al. 1991), which is dated as mid-Volgian (Fig. 5). Two specimens found in 1976 are also included in the study (juvenile PMO 220.401 and adult PMO 218.377). The adult was found at Agardhfjellet, also dated mid-Volgian, while the juvenile has been claimed to be Cretaceous, but both this and its locality is uncertain.

The geology and palaeontology of Svalbard, and of Agardhfjellet Formation have been subject to intensive research through many years (e.g. Nagy et al. 1988; de Buffr  nil & Mazin 1990; Dypvik et al. 1991; Fig. 5). Extensive exposed Jurassic deposits on Spitsbergen, Wilhelm  ya and Kong Karls Land are several hundreds metres in thickness (Vajda & Wigforss-Lange 2009).

The Jurassic was a warm and humid period globally, with relatively little variation across the latitudes, and ice-free polar regions. The CO₂ concentrations in the air were several times higher than they are today. Pangaea became fragmented, an event that led to a general sea level rise. Sedimentary successions from Scandinavia, Greenland and Svalbard show several transgressive and regressive episodes, which are probably linked to local tectonism (Vajda & Wigforss-Lange 2009). This can be seen in studies of the Janusfjellet subgroup, also showing that Slottsm  ya member has a maximum flooding interval in the lower half of the member (Reolid et al. 2010). The Barents Shelf was for a long time a stable platform, only to a small degree affected by the rifting further south. Seafloor spreading in the mid-Atlantic, connected to the break-up of Pangaea, progressed northeastwards during the Jurassic. In the Late Jurassic tectonism caused rifting between East Greenland and Norway and the development of a continuous rift from the North Sea to the Barents Sea (Vajda & Wigforss-Lange 2009). In the mid- to late Mesozoic Svalbard was situated at a latitude of around 70 degrees north (Ditchfield 1997). There was an open sea between northwest Europe and the Arctic in the Late Jurassic, after it had been closed and reopened again in the Callovian, but tectonic activity and a sea-level fall resulted in faunal provinciality (Dore 1991).

In the Late Jurassic several anoxic events occurred in northwestern Europe and the Arctic, together with deposition of organic-rich shales, which are found today throughout the area, in West Siberia and in North Alaska (Dore 1991). The Slottsm  ya Member consists of

mainly dark gray to black shales (commonly paper shales), with some discontinuous silty, sideritic beds and sideritic and dolomitic concretions. The organic carbon content varies between 1,5% and 12% (Nagy et al. 1988). Bioturbation is common higher up in the section, and terrigenous remains are preserved in several horizons. Some beds represent possible storm deposits (M. Collignon, pers. comm.). The Agardhfjellet Formation has been interpreted as an open marine shelf with low oxygen conditions, the Slottsmøya member as a deep shelf with offshore bars (Nagy et al. 1988; Dypvik et al. 1991). The area is argued to have been some hundred kilometres east or south of the shoreline (Dypvik et al. 2002). Sea temperature was relatively low. From belemnite stable isotope data it has been found to be 7,6°C in the Callovian and 8,1 in the Valanginian (Ditchfield 1997) The ecosystem seems to have had a relatively low diversity, interpreted from ammonite diversity (K. Hryniewicz, pers. comm.). Methane seeps, found by the Spitsbergen Jurassic Research Group, might have influenced the ecosystem (Hammer et al. 2011).

Since 2004, a high-latitude marine fauna has been discovered in this locality (Fig. 4) and in surrounding areas, consisting of ichthyosaurs, pliosaurs, plesiosaurs, seeps (Hammer et al. 2011), and invertebrates (e.g. a very interesting echinoderm fauna, see Rousseau 2011). Six field seasons have revealed one of the richest sites for Jurassic marine reptiles worldwide (Hurum et al. 2008).

This study

Plesiosaurs are well known from the fossil record, but many questions still remain, both on their phylogeny, ecology, distribution, locomotion, physiology, ontogeny and growth (see summary in Druckenmiller 2006). For knowledge about growth pattern and growth rate ontogenetic studies are needed (Klein 2010). Histological studies can be used to shed light on some of these questions and in the present work it has been used to understand growth and adaptation to marine life.

There is a need for more studies on reptile bone internal gross structure and microstructure. Even though bone growth is important, little is known of the postnatal limb development in reptiles (Farnum 2007), and very few studies have focused on sauropterygian bone histology (Wiffen et al. 1995; Klein 2010), especially the perichondral bone (Caldwell 1997b). Wiffen et al. (1995) proposed a hypothesis on ballasting and lightening in plesiosaurian limbs based on microstructure, and wrote that testing of this hypothesis is possible “since paleontological material from the Upper Jurassic or Lower Cretaceous would

allow a reconstruction of the ontogenetic trajectory of the closer ancestors of our Upper Cretaceous fossils” (Wiffen et al. 1995: p. 637) The Jurassic plesiosaurs in this study are therefore most suitable.

That the propodials of plesiosaurs have an inner cone structure is known, but to gain a clearer evolutionary explanation of the distribution of these structures, and of the distribution of secondary ossification centers in fossil tetrapods more work is needed (Carter et al. 1998). Other knowledge that might be gained from the gross internal structure are the patterns and sequences of ossification, which are “important sources of information that answer problems regarding development and evolution in the limbs of plesiosaurs” (Caldwell 1997a: p. 295).

Bone can be treated as an organ or a tissue, including or excluding the marrow, fat and interstitial material (Tont et al. 1977). A limb bone is not working in isolation: it communicates with other parts of the body through muscles, nerves and the vascular system to manage hunt for food, reproduction and other life functions. The shape and weight of the bone are worked on by evolutionary forces to obtain the best possible conditions for buoyancy and movement. For fossil material only the hard tissue is available for study. It is therefore difficult, but none the less important, to focus on the bone as an organ, for instance through comparisons with extant animals and by investigation of such features as the vascular system, muscle scars and possible fat content. Microstructure studies through the use of thin sections might give only a 2D perspective (naturally – the samples are flat!), which will lead to loss of understanding.

The goal in this study is to keep the perspective of a bone as a 3D organ interacting with other organ systems. Thus in this study thin sections in various directions are studied and combined with information on the gross internal structure gained from cuts through the entire bones. The aim is to give a description of the gross internal structure and the microstructure of fossil limb bones of four Volgian plesiosaurs from Spitsbergen, filling in a gap in our knowledge. The specimens are of different ontogenetic status, and different taxa, so that comparison must be treated with caution.

Material and methods

Material

The sample for this study are four plesiosaurs from Svalbard. Two are collected in the 2009 field season, PMO 216.839, which is a juvenile *species A* (Figs. 7B and 8A, B) and PMO 216.838 (Fig. 7A), which is a subadult *species B*. The two other are collected in 1976, PMO 220.401, a juvenile *species C* (Figs. 8E, F) and PMO 218.377, an adult *species D* (Figs. 8C, D). See table 1 and figure 6 for which bones are used from the different specimens.

The bones from PMO 216.839, PMO 220.401 and PMO 216.838 were found disarticulated, but together with several other bones from the same specimens. For PMO 220.401 it is not known whether the bones belong to a fore- or hind limb. The bones from PMO 218.377 originate from an articulated limb that is probably a hind limb. These specimens, together with the thin sections, are kept at Natural History Museum, University of Oslo.

Propodials and phalanges were used in this study because they are long bones and therefore best keep the record of the bone's history due to little remodeling. Growth marks might also be easier to observe (Botha & Chinsamy 2000). Mesopodials and the metapodium were included in the study because their microstructure has not been previously studied for plesiosaurs, and to map possible differences to the phalanges. The metapodium from PMO 220.401 showed ring-like structure when broken in two while being excavated in the field. This feature was not observed in the other bones studied, and also because of this, bones from this specimen were included in the study.

Comparison between the different specimens must be done with care, since each vary both according to their ontogeny and phylogeny. There are also many different factors influencing the visible bone structure in any animal. As in all such palaeontological studies, the limited amount of material makes it impossible to make definite conclusions about any larger group, such as all Jurassic plesiosaurs.

For comparison to gross internal structure, a radius from an extant undetermined mysticete (PMO 220.788) and the right humerus from an extant leatherback turtle (PMO 220.789) were used (Fig. 3). These are also kept at Natural History Museum, University of Oslo.

Methods

The bones were freed of all matrix and glued together with epoxy glue (Araldite). Casts were made in order to study shapes and provide possible exhibition material before the originals were sectioned.

In the two propodials, a longitudinal pre-postaxial cut was made using a water-lubricated slab saw (Diamant Boart) with a diamond blade. One of the halves from each of the bones was then cut longitudinally, but this time dorsoventrally. The result was one half and two quarters of each propodium. The half and one of the quarters were used for gross internal structure study. This was done by grinding the longitudinal pre-postaxial, inner side of the half, the dorsoventral inner side of the quarter and a transverse section from the last quarter on a lapidary wheel (Struers RotoPol-35) with 120 and 600 grit diamond abrasive discs (Struers MDPiano). The ground sides were then scanned (CanoScan 5600F) using rectified spirit between the fossil bone and the glass. Together the three scans provide a 3D understanding of the gross internal structure in these fossil bones (Fig. 9).

The whale and the turtle bones were cut on a band saw (Aigner) in the same way as the plesiosaur bones, resulting in one half and two quarters, together giving a 3D picture. The turtle bone pieces were ground on a wet grinder using 120 grit (Struers Knuth Rotor) before being scanned. The whale radius was photographed (Canon Digital Ixus 95 IS) (Fig. 3).

The last quarter from each of the two plesiosaur propodials was used for making thin sections. Six pieces from different locations in the bone were taken out (Figs. 9A, D). Transverse sections from the midshaft (neutral) region and longitudinal sections from the articular regions were included to ensure the best available information about both ontogeny, growth in length and structural orientation of trabeculae (Felts & Spurrell 1965; Chinsamy-Turan 2005). Transverse sections might be misinterpreted if seen isolated, due to the gross internal structure. Therefore, thin sections in all three directions were made (Fig. 9A, D). The pieces were cut, ground on a lapidary wheel (Struers Discoplan cutting and grinding machine), impregnated with epoxy (EpoFix) and mounted on glass before being ground to the right thickness (Thorlag thin section machine). The first thin sections made were 30 - 35 μm and the later 50-60 μm . The increase in thickness was done to investigate whether more structures would appear.

From the phalanges, the mesopodials and the metapodium thin sections were made. A transverse cut was made through the bone, followed by a longitudinal dorsoventral cut through one of the resulting halves. Thin sections were then made, following the procedure

described for the propodials, one from a transverse section and one from a longitudinal section for each of the bones, and in addition a longitudinal pre-postaxial section for PMO 220.401 (Fig. 8).

All thin sections were studied under a microscope (LeicaDMLP) on 2-100x magnification and photos were taken using a LeicaDC300 camera connected to the microscope and computer software (ACDSee 6.0) (Figs. 11-14). The thin sections were also scanned to show the overall structure, using a Nikon Super coolscan 4000 ED scanning device and Nikon Scan 3.1 software (Fig. 10).

Articulated limbs from two of the same plesiosaur specimens were used for taking measurements of the distance between the propodials, epipodials and mesopodials to obtain information of the possible existence and size of cartilage caps. For PMO 216.839 and PMO 216.838 these measurements were taken on hind limbs. For PMO 218.377 and PMO 220.401 such measurements were not possible.

Terminology

In bone microstructure research the terminology used by different workers is conflicting and not always well defined. The microstructure in this study was described using the terminology of Reid (1996) except that “longitudinal canals” is used here for describing what is called “parallel canals” in that work. “Peripheral rest lines” is used as described in Chinsamy (1990).

It is not yet clear which species the specimens in this study belong to. The specimens are all thought to belong to different species, and are thus named *species A-F* in this work. Work is in progress to resolve the question, through a Ph.D. thesis from the Spitsbergen Jurassic Research Group (E. M. Knutsen, pers. comm.).

Institutional abbreviations

PMO: Palaeontological Museum Oslo (now a part of Natural History Museum, University of Oslo). SVB: Svalbard Museum, Longyearbyen.

Abbreviations used in the table and figures

C= carpal, **c** = cortex, **d** = decomposition, **F**= femur, **H** = humerus, **l**= lamellar bone, **m** = medulla, **Ms**= mesopodium, **Mt**= metapodium, **n** = nutrient artery, **o** = osteocyte lacunae, **P**= phalanx, **p** = peripheral rest lines, **po** = primary osteon, **r** = resorption cavity, **so** = secondary

osteon, **T**= tarsal, **t** = area with collapsed trabeculae, **v** = simple vascular canals, **vs** = vascular system (unspecified), **w** = woven bone, **y** = medullary cavity, **z** = cartilage cap.

Results

PMO 216.839

Gross external anatomy

The humerus (Fig. 7B) is 18 cm long, and has neither clear facets nor a well-defined head and has quite similar pre- and postaxial sides. The carpal (Fig. 8B) is 3 cm long and almost as wide, and has a dumb-bell shape on one side. It might have been compressed during preservation. The phalanx (Fig. 8A) is as long as the carpal, but narrower, and has the typical dumb-bell shape of a long bone.

Gross internal structure PMO 216.839 humerus (Figures 9D-F)

The juvenile humerus has an inner structure of two cones whose apices do not meet, being separated by a small medullary cavity. The cones were made up of endochondral bone. In the present bone, the area is mainly infilled with clay mineral and has only small isolated patches of collapsed trabeculae left. It is thus clear that the cones were made of a much weaker building material than the rest of the bone.

Outside the cones is a sheath of periosteal bone, shaped like flattened triangles in a longitudinal section. They also seem to make a bridge through the bone around the medullary cavity, separating the two cones completely. The periosteal sheath does not cover the whole length of the bone and covers different amount on different sides of the bone, exemplified by being absent the first 1/6 of the length from the proximal end on the anterior side while covering almost the entire length at the posterior side. For the distal end such a comparison cannot be done due to the fact that a part of the bone is missing posteriorly.

The vascularization is quite abundant. In the endochondral cones, many large canals lead from the medullary cavity and longitudinally towards the distal end of the bone. The proximal part is less organized. In the periosteal parts, the canals run longitudinally distally to the medullary cavity, and less organized in the proximal parts. In the middle of the anterior periosteal triangle, a large canal intrudes the bone towards the area around the medullary cavity. This is probably the nutrient artery.

The periosteal area is overall slightly denser than the endochondral, with the most cancellous parts being the areas distally and laterally. For the endochondral region, the distal part is quite cancellous, more than the proximal end. In the epiphysis there is no sign of a

secondary ossification center. On the outside of the bone, the proximal epiphysis shows several small pits, a few millimetres in diameter. With the present material it is not possible to tell whether also the distal end had these pits.

Microstructure PMO 216.839 humerus. (Thin sections 216.839I-VII. Bone figure 7B, microstructure figures 11A, B and E-G)

The bone is divided in two: the cortex, made from periosteal bone (Fig. 11A), and the medulla, probably from endochondral bone (Fig. 11E). In the medullar region the bone is almost completely exchanged for clay mineral in all the thin sections, mixed with smaller parts of collapsed trabeculae with some primary osteons. The periosteal parts are much better preserved and were probably consisting of a more robust material, being ossified completely. The cortex does not show any growth marks. The periosteal bone is woven (Fig. 11B). Distally it is more compact in outer parts, but in proximal parts, the opposite is true. Outmost there is a band with light colour containing collapsed trabeculae. The reason might be that the bone was still growing and that this part therefore had less ossified material.

The cortex is well vascularized. In parts distal to the mid-shaft the vascular direction is strongly longitudinal (Fig. 11A). Close to the proximal epiphysis the periosteal is more cancellous and the canals run in all directions, but a majority still longitudinal. The canals are mostly primary vascular canals not yet surrounded by lamellar bone, in addition to some primary osteons. There is a large size variation among the canals (Fig. 11G). No secondary deposition or remodeling seems to have taken place. The bone shows several signs of decomposition especially in the outer edge area (Fig. 11E), both a narrow band of small dots a few millimetres from the edge and what might be traces of burrowing bacteria or fungi.

Microstructure PMO 216.839 carpal. (Thin sections 216.839X and XI. Bone figure 8B, gross structure figures 10A and B, microstructure figures 11C and D)

The carpal does not have a real dumb-bell shape, but one of the lateral sides is restricted in a similar manner to bones with a periosteal sheath (Fig. 8B). In transverse section there is no cortex, but in longitudinal section, the bone shows an inner cone-like structure comparable to the one in the propodial and in the phalanx (Fig. 10B). The inner cone structure is similar to the one in the propodial, showing collapsed trabeculae and clay mineral infill. Overall, the sections have quite low quality and thus low information content.

In transverse section the bone has two different parts, one dorsal and one ventral, with a band consisting of collapsed trabeculae and clay mineral infill in between (Fig. 10A). The assumed dorsal part covers more than half of the section. It has radiating trabeculae, especially on one lateral side, while on the other side, the trabeculae are thicker and have no apparent direction (Fig. 11C). The assumed ventral side shows no orientation of the trabeculae. It covers only a half circle. The bone is woven (Fig. 11D). The longitudinal section is quite similar to the phalanx. It contains a more visible cone, in which the trabeculae are collapsed. In the outer parts, the trabeculae are larger, more complete and the bone is very cancellous. The vascular system is mostly consisting of simple vascular canals in a reticular pattern. A few primary osteons are visible in the inner bone in longitudinal section, and some in transverse section. There are no secondary osteons or other traces of remodeling.

Microstructure PMO 216.839 phalanx. (Thin sections 216.839VIII and IX. Bone figure 8A, gross structure figures 10C and D)

The phalanx has a typical dumb-bell shape (Fig. 8A), which means it has a periosteal sheath around the middle. It does not show a compact cortex, the outer parts are instead cancellous. When seen in longitudinal section, a cone similar to the one in the carpal is visible, but not as well defined. The inner structure of the cone is collapsed, showing only smaller bone pieces in clay mineral infill. In transverse section not much information can be obtained. The middle part of the section is collapsed. As the carpal and the propodial from this specimen, the preservation is not very good and the sections show several signs of decomposition.

In transverse section a large area is consisting of similar collapsed trabeculae and shale as in the longitudinal section (Figs. 10C, D). Around the outer edges of the bone, the trabeculae are more complete, and the bone is cancellous. Some primary osteons can be seen in the outer parts, together with simple vascular canals and some possible resorption cavities. The main vascular direction seems to be longitudinal. In the cone one primary osteon appears. There are no signs of remodeling or growth marks.

PMO 220.401

Gross external anatomy

The mesopodium (Fig. 8F) is almost circular and flattened, with a diameter of 3,5 cm. The metapodium (Fig. 8E) has a dumb-bell shape and is 4,5 cm long.

Microstructure PMO 220.401 mesopodium. (Thin sections 220.401III-V. Bone figure 8F, gross structure figures 10E and F, microstructure figures 12B-D)

This bone does not have a shape that indicates the presence of periosteal bone (Fig. 8F). Inside there is no compact cortex and the bone is porous throughout. The inner structure is quite similar to the adult mesopodium (PMO 218.377) looking like a “sandwich”, but in this one there are cone shapes in longitudinal section (Figs. 10E, F). The vascular system consists of simple vascular canals that are not organized in “rings” as observed in the metapodium. There is, however, a tendency for the trabeculae to radiate diagonally in some areas. The inner part of the bone consists of collapsed trabeculae, with clay mineral infill in the epiphysis (Fig. 12B). As for several other bones, degradation of some kind has happened in the middle of the trabeculae. There are no traces of remodeling. In one area in the outer part there is an extremely high density of osteocyte lacunae with visible canaliculi (Fig. 12C).

The longitudinal pre-postaxial section has three different areas: the outermost, partly decomposed and with clay mineral infill, a middle area that is very porous and with no apparent organization and the innermost area which is more compact with numerous small vascular canals (Fig. 12D). The bone also has two large cavities, one probably leading into a medullary cavity and the other another large blood vessel.

Microstructure PMO 220.401 metapodium. (Thin sections 220.401I and II. Bone figure 8E, gross structure figures 10G and H, microstructure figure 12A)

The bone has a dumb-bell shape, and thus a periosteal component (Fig. 8E). There is no compact cortex, and the bone is porous throughout (Fig. 12A). In the middle part and in five broad “stripes” leading in to it, the trabeculae are collapsed (Fig. 10G). In longitudinal section, the bone has a cone inside, consisting of collapsed trabeculae, while the areas around it are almost complete (Fig. 10H). The epiphysis of the bone has clay mineral infill, like several of the others, with an increasing amount of collapsed bony trabeculae inwards.

The most striking feature is that the trabeculae in the outer parts are organized in “rings” (Figs. 10G, H and 12A). In a very ordered fashion, trabeculae are laid down with

equal distance between them. This is apparent on all sides of the bone, except where the trabeculae are broken. In the outermost row, the cavities that probably contained blood vessels are smaller and more circular (Fig. 12D). The vascular canals are mostly simple canals and numerous, with a few primary osteons in transverse section closer to the middle. One large canal leads from the outside and in to the middle of the bone. There are no signs of remodeling. The trabeculae show the same degradation in the middle (Fig. 12A) as in many other specimens in this study. In the outer parts in longitudinal section many osteocyte lacunae are visible.

PMO 216.838

Gross external anatomy

The femur (Fig. 7A) is 39 cm long, and has a different shape from the juvenile humerus, being non-symmetrical on the pre- and postaxial sides. It has clear facets on the distal epiphysis and a well-developed head and trochanter proximally.

Gross internal structure PMO 216.838 femur. (Figures 9A-C)

In the subadult femur the two endochondral cones are also clearly visible, and they occupy a relatively larger part of the bone than in the juvenile. The distance between the two apices is larger. A structure which seems to be the medullary cavity appears between the cones. In this specimen the preservation is better than in the juvenile, and there are complete trabeculae throughout the endochondral cones to the epiphyses, indicating that ossification is completed. The bone lacks a part distally, on the posterior side, and in the middle, above the distal cone. This makes it difficult to be sure about the size of the medullary cavity and the possible presence of a nutrient artery, but there might be traces of a nutrient artery, similar to the one in the juvenile, on the posterior side.

The periosteal sheath does not go all the way to the epiphyses, but covers only the middle third. The periosteal tissue is more compact than in the juvenile and much more compact than the endochondral bone. It seems to have three zones; one inner, dark brown, almost purple, which is very compact; then a lighter, compact band; and a more cancellous area outermost, blending in with the endochondral part close to the epiphyses. The vascular organization in the periosteal sheath must be investigated through the use of thin sections (see below), due to the compactness and the dark colour.

In the endochondral region, some of the larger vascular canals have a longitudinal direction, but it is mostly consisting of trabeculae in a non-orderly fashion, especially in the proximal part. In the distal endochondral cone there are some large cavities, close to the middle, all pointing proximal-distal, but mainly there is no preferred trabeculae direction.

Microstructure PMO 216.838 femur. (Thin sections 216.838I-VII. Bone figure 7A, microstructure figure 13A-G)

Two distinct parts of the bone are visible: the outer, very compact cortex from periosteal bone, and the inner medullary region consisting of cancellous endochondral bone (Figs. 13B, C, D, G). The cortex varies in thickness depending on where on the propodium the thin section is taken, due to the inner cone structure.

The cortex itself has two regions. They appear as three in a photograph (Figs. 9A, B), but thin sections reveal that there are two: one inner, seemingly darker on a scan, but lighter in a thin section, and one outer, darker band (c1 and c2 in Fig. 13A). Two different texture types are present in the cortex, both woven bone (Fig. 13D), but the outer has larger fibres that in many places form a regular network. The outer edge shows signs of decomposition, probably made by bacteria or fungi. There are no growth marks, and the bone is azonal. The outermost part contains several resorption cavities, and a few primary osteons (Fig. 13A). In the inner part of the cortex there are no resorption cavities, but abundant secondary osteons that increase in density inwards, however not making up compact Haversian bone. Some of the secondary osteons are completely infilled, and between them are a few primary osteons (Figs. 13A, B). The vascularization in the cortex is mostly longitudinal, with a few short canals running radially, connecting the longitudinal canals (Figs. 13A, G). Proximal to the medullary cavity the cortex is remarkably more compact and less vacularized than sections distal to the mid-shaft. Secondary osteons are more abundant proximally.

The cavities in the medulla are large and the trabeculae show clear signs that remodeling has occurred, being reconstructed cancellous bone (Figs. 13C, G). Secondary osteons are abundant, some of them wholly filled, and osteocyte lacunae are very abundant compared to the cortex (Fig. 13C). A few larger canals also appear in the medulla, more frequently in the distal half of the bone. One of these has lamellar bone on one side and woven bone on the other.

Both of the epiphyses have larger cavities and are more cancellous than sections taken from the shaft. In the proximal epiphysis area the endochondral cancellous medulla extend to

the articular surface. The outermost millimetres are a bit more compact than the inner, and the most proximal trabeculae all lie parallel to the proximal epiphysis and have experienced very little remodeling (Fig. 13F). The distal epiphysis also has similar trabeculae extending to the epiphysis. It is more compact than the proximal epiphysis. In the outermost part some decomposition has happened (Fig. 13E). One larger canal is present, but it is uncertain from the section whether it reaches the outer surface.

There is no calcified cartilage in any part of the bone. In the middle of many trabeculae, both in the medulla and the cortex, some form of decomposition has happened, in the medulla appearing as small bubbles (Fig. 13D).

PMO 218.377

Gross external anatomy

The tarsal (Fig. 8D) is shaped like a trapezoid (5 x 4 cm) with with five sides. The phalanx (Fig. 8C) is dumb-bell shaped and 3 cm long.

Microstructure PMO 218.377 tarsal. (Thin sections 218.377III and IV. Bone figure 8D, gross structure figures 10I and J, microstructure figures 12E and F)

The external shape of the bone might indicate that there is some periosteal bone on one side (Fig. 8D). On the epiphysis of the bone, pits where inner vascular canals came out are observed. In transverse and longitudinal section, the tarsal has quite similar overall structure: a band in the middle with small pieces of collapsed trabeculae, and cancellous bone on both sides reaching to the outer edges (Figs. 10I, J). There is no compact cortex.

In the middle of the transverse section the band of collapsed trabeculae makes up approximately half of the width and is surrounded by cancellous bone. A few osteocyte lacunae are scattered randomly. One very large canal with a triangular shape comes in from the side almost in the middle of the bon. This is probably the nutrient artery (Fig. 12E). The walls of this canal are lamellar, and originally avascular, but later they were being reconstructed into densely spaced secondary osteons (Fig. 12F). On the other side of the bone than the blood vessel, there is a small, more compact area, where the outside shape indicates that there might be periosteal bone. In this area, faint lines, which might be peripheral rest lines are visible.

In longitudinal section the broad band with broken trabeculae in the middle is clearly visible, and is more compactly filled closer to the epiphysis. Surrounding the middle band the bone consists of very remodeled trabeculae, of which some show cracks. On the sides, close to the middle of the bone are some more compact areas. The longitudinal section also shows some signs of decomposition. There is no organization of the vascular direction apart from a few larger longitudinal vessels. Only secondary osteons are visible throughout the bone (Figs. 12E, F), and the bone is azonal. In outer parts some resorption cavities are observed.

Microstructure PMO 218.377 phalanx. (Thin sections I and II. Bone figure 8C, gross structure figures 10K and L, microstructure figure 14A-I)

The dumb-bell shape indicates the presence of periosteal bone tissue (Fig. 8C). In transverse section the bone has an inner cancellous medulla and a slightly more compact, narrow outer part which probably consists of periosteal bone (Fig. 10K). The inner medulla has continuous trabeculae throughout, and turns more cancellous towards the center. It is strongly remodeled, and the trabeculae have lamellae that surround cavities of different size, of which some are wholly infilled (Figs. 14B, C, D). In transition to the cortex there are several small secondary osteons, often infilled. Some of the trabeculae in the medulla have a core where decomposition has taken place, as seen in several other thin sections (Fig. 14D).

The outer part is of a different texture, as in the PMO 218.838 femur containing some resorption cavities, secondary osteons and large, visible fibres (Figs. 14A, F). In the outermost part in transverse section, and in outer parts in longitudinal section, two to four lines can be seen. These are probably peripheral rest lines (Figs. 14A, E, G). They disappear almost completely in polarized light (Figs. 14H, I). They are not visible in all parts of the sections, probably due to the fact that the rim is attacked by bacteria or in other way decomposing in many places (Fig. 14G).

In longitudinal section, three large longitudinal canals are visible, reaching from the cancellous middle part and all the way to the epiphysis (Fig. 10L), but apart from these large canals, there is no apparent vascular organization. On the outside of the bone, small pits that the large vessels end in can be seen. Inside is a cone: the two bottom side areas on each side are much more compact in the shape of triangles, with the same texture as the outer edge in transverse section. In these areas, small secondary osteons are visible, several of them infilled. There are also several large fibres, in a messy pattern (Fig. 14F). A few osteocyte lacunae are

visible. The epiphysis has in some way been degraded: the outer parts are infilled and partly replaced by clay mineral.

Distance between limb bones in articulated specimens

The measurements of the distance between the bones in articulated limbs from the same specimens as the thin sectioned bones, show a difference between the juvenile (PMO 216.839) and the subadult (PMO 216.838). The juvenile measures 10-15 mm from the distal epiphysis of the femur to the epipodials, while the subadult bones almost touch each other (1-3 mm distance). From the epipodials to the first row of tarsals the distance is 10 mm in the juvenile and 1 mm in the subadult, while there is a larger distance from the first row of tarsals to the next in the subadult than in the juvenile.

Discussion

The gross inner structure is an adaptation to life in water

Large diversity in inner structure

The first obvious observation made from the gross internal structure in the present material, is that there is not one pattern of Svalbard plesiosaur limb bone (e.g. Figs. 9, 10 and 15). Easy conclusions drawn from single bones can be misleading. In this material there are both very compact and very porous bones and parts of bones, and many observations differ from the existing literature in the field. Bones that have been growing very close to each other often differ significantly.

It is difficult to compare the present material because it differs both in ontogenetic level and phylogeny, but some general observations may however be stated. In all the long bones, an inner cone structure can be seen, most pronounced in the propodials. The propodials also seem to have a tiny medullary cavity, while the rest of the bones are most porous in the middle, but with no open cavity. None of the bones have a secondary center of ossification or any other special structure at the epiphysis.

In the propodials there is a clearly defined cortex that is more compact than the medulla. This is also the case for all the other plesiosaur propodials from Spitsbergen where this can be observed, but that are not included in this study (pers.obs.). This is in contrast to many other marine animals, such as cetaceans, which show a regression or lack compact cortices (Figs. 3C, D; de Buffrénil & Schoevaert 1988).

Many of the bones do show large zones of collapsed trabeculae (e.g. Figs. 11E and 12A). Seen in transverse section, the zone of collapsed trabeculae might result from pressure during fossilization (Hua & de Buffrénil 1996), which is probably the case for some of the mesopodials looking like “sandwiches”. Differences in compaction is not uncommon: among plesiosaur remains in the Oxford Clay, highly compacted and uncompact bones from the same specimen are found close to each other (Martill 1991). The structures might however also reflect the growth pattern, a view that fits better with longitudinal section from the PMO 216.839 humerus and the other long bones that have the cone structure.

Porosity

Wiffen et al. (1995) are widely cited for their conclusions that plesiosaur bone became more cancellous through their life span, a view first emphasized by Williston (1903). One of the findings from the present work is that this conclusion is not valid for all plesiosaurs. Since the specimens in this study are not conspecific, it is not possible to be sure that the juvenile PMO 216.839 will not become more porous through ontogeny, but the PMO 216.838 material proves that not all adult plesiosaurs have bone that is entirely porous (Figs. 9 and 13A, B, G).

Caution also has to be taken if one intends to calculate porosity from the type or amount of cavities in an aquatic animal bone, and from that conclude on buoyancy or amount of blood supply. The cavities not only contain blood vessels in life of the animal, but also nerves, lymph system and other connective tissue (Starck & Chinsamy 2002), and marrow cavities in the medullar area contain water, ash, protein and lipids in marine animals (Higgs et al. 2011). Marine reptiles must have had marrow in the long bones, and possibly lipids for buoyancy (Kiprijanoff 1883b; Sheldon 1997; Kaim et al. 2008). Pyrite framboids found in fossil plesiosaur and whale bones have been seen as evidence for lipid content, but this is contested (see summary in Kiel 2008). In collaboration with the Spitsbergen Jurassic Research Group, work is in progress at the Institute for Energy Technology at Kjeller, Norway, investigating the possibility for preservation of lipids in the plesiosaur bones (J.Kihle, pers. comm.).

Inner endochondral cones

The two components of the bone that determine the growth and shape are the periosteal and the endochondral. In particular bones in some groups, among them several plesiosaur limb bones, the periosteal component is lost altogether (Caldwell 2002). The propodials, the phalanges and the metapodium in this study have a periosteal component. Internally, it is more clear in the propodials, but also in the phalanges there is another bone type in the outer parts that is more compact and contains osteons. This is periosteal bone (Figs. 9 and 10). The propodials, phalanges and metapodium are dumb-bell shaped (Figs. 7 and 8A, C, E), which means they had periosteal bone (Farnum 2007). It seems that the periosteal bone is almost completely lost in the mesopodials (Figs. 8B, D, F), apart from a small patch on one margin on some of the observed specimens. This is the same as found in another Late Jurassic plesiosaur, *Cryptoclidus* (Caldwell 1997a).

In the long bones, the relationship between the periosteal and the endochondral components make the gross inner structure of two cones (e.g. Figs. 9A, D and 10D, H). These cones are important to map in order to understand how such a bone grows, the evolution of growth of long bones (Carter et al. 1998), and a prerequisite for understanding other features of the plesiosaurian limb. Since the cones are shaped as pyramids and the periosteal sheath as a cylinder-shaped box around them, transverse sections taken from different places along the shaft will vary greatly in appearance, unlike cross-sections from mammalian tubular bone. This is important when measuring cortex thickness or porosity. With this in mind, there has been done surprisingly little work on the gross internal structure of plesiosaur bones, and the inner cones has not been described since the work of Moodie (1916; Fig. 2F-I). The present study covers some aspects but further studies are still needed.

The structure of two endochondral cones in propodials inside an outer sheath of periosteal bone has been described in several marine animal groups, among them extinct and extant marine mammals (the manatee *Trichechus latirostris*, Fawcett 1942; cetaceans, Felts & Spurrell 1965; the dolphin *Delphinus delphis*, de Buffrénil & Schoevaert 1988; early archaeocetes, Madar 1998) and extinct and extant marine reptiles (the leatherback turtle *Dermochelys coriacea*, Rhodin 1985; the sauropterygians *Pachypleurosaurus*, de Buffrénil & Mazin 1989; and *Placodus*, de Buffrénil & Mazin 1992) (Fig. 3). It has also been observed in plesiosaurs (Kiprijanoff 1883b; Lydekker 1889; Moodie 1908; 1916; Fig. 2B, D, F, G).

The cones are interpreted as endochondral bone (Moodie 1908, 1916). In evolution of the ossified endoskeleton in vertebrates, perichondral ossification evolved before endochondral ossification (Carter et al. 1998). There are two main patterns of endochondral ossification: the well-organized, efficient process in mammals and many extant lizards, and the process in basal tetrapods, dinosaurs, extant crocodiles, turtles and birds. In the latter group, during prenatal development, perichondral ossification advances towards the bone ends faster than endochondral ossification, unless bone growth is relatively slow (Haines 1969; Carter et al. 1998). This leads to large "cartilage cones" being trapped inside the bone shaft, before erosion of the cartilage and endochondral ossification happens later in development, often quite slowly. It is common that calcified cartilage fragments are "trapped" in trabeculae for a while (Haines 1969). Chondrification and ossification are often decoupled, so that selection can act on them independently, a feature that has lead to many different inner bone designs in reptiles (Farnum 2007).

Several workers found calcified cartilage in trabeculae in propodials. In a Late Cretaceous elasmosaur, juvenile globular calcified cartilage is “the main component” in the medulla in both humerus and phalanx (Wiffen et al. 1995), and in a subadult elasmosaur and *Lütkesaurus* (not valid taxa) from the late Cretaceous, small areas in trabeculae in the medulla were found (Kiprijanoff 1883a; Fostowicz-Frelik & Gazdzicki 2001). In adult specimens, small remnants have been found in a Late Cretaceous elasmosaur (Wiffen et al. 1995). These observations, even though limited in number and in age, confirm a decreasing amount of calcified cartilage in the endochondral medulla through ontogeny due to increasing ossification of the bone. The middle of trabeculae many places in the bones in this study show some form of change (Figs. 11E, 12A, 13D and 14D). This is not calcified cartilage, but probably signs of decomposition. It is a sign that the middle areas of the trabeculae were different from the edges, probably because many of them still contained calcified cartilage.

In the Svalbard specimens cones are observed in both juvenile, subadult and adult specimens, indicating that they persist through the life of the animal. The only adult plesiosaur that is sectioned (Moodie 1908) is said not to have kept the cones, but this would need to be reinvestigated. The propodial cones observed in the present study differ between the ontogenetic stages. In the juvenile specimens the cones seem to consist of a weaker material than the surrounding bone. They are often collapsed when found (Fig. 1) and in thin sections they consist of scattered pieces of collapsed trabeculae and clay mineral infill (Figs. 11E and 12B). This could be diagenetic, but it is unlikely that diagenesis would give this particular structure of two cones if the bone was subject to a random process. How well vertebrate bones resist compaction during fossilization depends on internal composition, thickness of cortical bone and the sediments surrounding it (Martill 1991). This means that areas that are not completely ossified and that consists partly of cartilage could collapse easier than areas consisting of bone. Cartilage is more flexible and elastic than bone, but is a little less resistant to compression and less resistant to tension and shear (Liem et al. 2001). The subadult femur has complete trabeculae throughout the medullary area and is not collapsed, probably because it has undergone complete ossification.

For the plesiosaurs in this study, the “cartilage cone process” seems very plausible, but with a twist: there are cones in all the long bones, and no free medullary cavity, meaning that the erosion did not happen as described above. This is probably because plesiosaurs were marine animals and seen also in the leatherback turtle (Fig. 3A; Rhodin 1985). The propodials both have a small medullary cavity restricted to the middle of the bone, which is relatively

smaller in the subadult (Fig. 9A compared to 9D), while the other bones seem to have trabeculae throughout (Fig. 10). This is the same as other workers have found. Moodie (1908, 1916) reports that the medullary cavity is small in an embryo, remaining only partly in subadults and disappearing completely in adults. Some Cretaceous species have medullary cavities filled with trabeculae (Kiprijanoff 1883b; Salgado et al. 2007).

The ichthyosaur *Omphalosaurus* had a small medullary cavity, while *Stenopterygius* and *Ichthyosaurus* did not (de Buffr  nil & Mazin 1990). Cetaceans and large marine turtles also lack an open medullary cavity and cancellous bone is widely distributed in most medullary areas of turtle and crocodilian long bones (Figs. 3A, C; Enlow 1969). The altered buoyancy in water suppresses the weight-bearing role of the skeleton, and this will alter the histology. The lack of a free medullary cavity might be an epigenetic reaction to an aquatic environment and the mechanism behind it an imbalance between resorption and reconstruction (de Buffr  nil & Schoevaert 1988; Wiffen et al. 1995). The internal resorption in the dolphin humerus create and maintain a complex network of trabeculae, whereas it completely remove them in the tubular bones of terrestrial mammals (de Buffr  nil & Schoevaert 1988). See further discussion on remodeling below.

Microstructure might reveal physiology and growth pattern

Ontogenetic status

Based on external characters the specimens in this study are argued to be two juveniles (PMO 216.839 and 220.401), one subadult (PMO 216.838) and one adult (PMO 218.377). Study of the inner features of the bones confirms this: there are no traces of remodeling in PMO 216.839 and 220.401 (Figs. 11A and 12A), while PMO 216.838 and PMO 218.377 both have been remodeled and have a cortex with secondary osteons (Figs. 12E and 13B). If the lines visible in the PMO 218.377 phalanx are peripheral rest lines (Figs. 14A, E, G) this would indicate that it is a fully grown specimen.

Vascularization

Vascularization is the means of transport of blood and nutrients to the bone, and it changes throughout the life of an animal. Embryonic limbs are supplied by a complex network of many blood vessels. During growth one of these becomes the main artery leading into the

limb in the adult, called the brachial (to humerus) or femoral (to femur). It is not derived from the same vessel in every case (Wake 1979). In turtles the brachial splits in the radial and the ulnar artery and then again into digital arteries (Wyneken 2001).

In the Svalbard propodials, the main organization of the vascular system in the cortex is longitudinal, with a few additional canals connecting them (e.g. Fig. 11A, F, and 13A, G). The longitudinal pattern is more pronounced distally to the mid-shaft. In other bones than propodials, the vascular system is less organized. In the literature the description of plesiosaur vascular network include all possible organization except from laminar, and it is often unclear which is actually found and which definitions are used. A longitudinal pattern is found in other plesiosaurs, sometimes in the cortex but more often in the medulla (Kiprijanoff 1883b; Gross 1934; Wiffen et al. 1995). To overcome the use of subjective and confusing terminology, methods for quantifying bone vascular orientation mathematically can be used (de Boef & Larsson 2007). It is easier to reach a conclusion about the amount of vascularization. All workers in the field report on a moderate to high vascularization of the cortex, which fits with the present material. Without it being calculated, the juvenile propodial seems more heavily vascularized than the subadult (Figs. 9, 11A and 13A). The abundant vascularization, also in subadult and adult specimens, is contrary to many reptiles, which often have avascular bone. Dinosaurs are an exception (Currey 2002).

In addition to internal vascularization in the limb bones, the relationship with the rest of the vascular system is important. A large nutrient artery can be seen in the juvenile humerus, probably in the subadult femur, and in the adult tarsal (Figs. 9D, 10I and 9A). It has also been observed in subadult Cretaceous plesiosaur *Ogmodirus martinii* (Moodie 1916). Some workers found what they described as four large vascular canals leading into the middle of the bone, in juvenile specimens, a feature that was not observed in adult specimens (Williston 1903; Moodie 1908). In the specimens in this study, some larger canals are visible in the medulla in the subadult propodial, but not in the same pattern as described by the other workers.

In the present material many of the bones, both propodials, mesopodials and phalanges, and both juvenile and adult bones, show small pits on the outside epiphysis. On the phalanx of PMO 218.377 pits correspond to large vascular canals inside that run longitudinally from the middle of the bone. This has been observed before, in subadult plesiosaurs (Moodie 1916; Fostowicz-Frelik & Gazdzicki 2001), and also in ichthyosaurs from Spitsbergen (de Buffrénil & Mazin 1990). Going through the epiphysis, these blood

vessels would enter the cartilage cap, which the plesiosaurs probably had (Kiprijanoff 1883b; Brown 1981). Measurements of the distance between the different limb bones in articulated specimens show a larger available space for this cartilage in the juvenile PMO 216.839 than for the subadult PMO 216.838, the latter having virtually no space for this between the propodials and the epipodials, but more space between other bones.

Leatherback turtles show a similar feature. They have different bone growth in the physeal area from other turtles, and one of the main differences is that there are large vascularized cartilage canals traversing the growth plate and entering the cartilage of the epiphysis (Rhodin 1985). Leatherback turtles are the only turtles and the only extant reptile which show this feature. In the fossil record some protostegid fossil turtles show the structure. Other turtles do neither have transphyseal vascularization in juveniles nor in adults (A. Rhodin, pers.comm.). Rhodin (1985) hypothesized that the so-called transphyseal vascularization is not due to large size, but to rapid growth to a large size, where the vascularization sustained the high metabolic requirements of fast-growing cartilage. The small pits might be an indication of rapid growth in plesiosaurs.

Fibrillar organization and tissue type

Amprino's rule says that growth rate influences fibrillar organization and tissue type, and this has been used to deduce growth rate from bone microstructure. However, this is contested and there is uncertainty about the relationships between growth rate, fibrillar organization and tissue type (e.g. see review in Cubo et al. 2005; Montes et al. 2010).

In areas where it is possible to observe fibrillar organization in the Svalbard material, there is woven bone (e.g. Figs. 13D and 11B). This has also been found in other plesiosaur propodium and phalanx material (Wiffen et al. 1995; Fostowicz-Frelik & Gazdzicki 2001). The only lamellar bone found is in the adult tarsal (PMO 218.377) around the nutrient artery, and possibly indicates slow growth in that area. Woven bone might indicate fast growth and is often used as proof of relatively rapid growth, in contrast to lamellar bone that grows slower.

Not many workers describe the tissue type in plesiosaur bone. Wiffen et al. (1995) found dense Haversian bone in an adult Cretaceous elasmosaur, and Salgado et al. (2007) found even more of it. With the present material this cannot be confirmed. It seems not to have Haversian bone, either because the specimen is a subadult, or because it had yet to evolve. Haversian bone is recognized as an advanced bone tissue (de Ricqlès 1976).

An interesting question is whether plesiosaurs had fibro-lamellar bone, which is considered to be a fast-growing tissue compared to lamellated and zonal bone. A phylogenetic analysis based on histology in other sauropterygians indicated that plesiosaurs should have fibro-lamellar bone (Klein 2010). The material from Svalbard is not really suited to solve the problem, but it seems to have woven bone, secondary osteons and many osteocytes, as well as being well vascularized and mostly azonal, all being characteristics of fibro-lamellar bone.

Remodeling

In the studied material, the subadult and the adult specimens are clearly more remodeled than the juvenile, which shows no remodeling (Figs. 11A and 12A in contrast to Fig. 12E and 13B). There are secondary osteons in the cortex of the subadult and adult, which is not common in crocodiles and many other reptiles (Chinsamy & Dodson 1995). De Ricqlès (1976) stated that osteons found in nothosaurs and plesiosaurs often are only primary even though they look like secondary osteons because the whole cortex is not reconstructed. In the present material (PMO 216.838 and 218.377) there are real secondary osteons, especially in the inner part of the cortex. Secondary osteons in plesiosaurs are also found in other studies (Kiprijanoff 1883a; Seitz 1907; Wiffen et al. 1995; Fostowicz-Frelik & Gazdzicki 2001; Salgado et al. 2007).

In leatherback turtles there is a lack of internal remodeling allowing the cones to remain well differentiated throughout life (Fig. 3A; Rhodin 1985). In the Svalbard plesiosaurs remodeling has happened in the subadult and adult specimens, but not very extensively, so that both the periosteal and the endochondral parts are kept more or less in what appears to be their original position.

Growth pattern

An important feature in the present material is the absence of zonation in most of the bones (Figs. 9, 11A, 13A and 14A). This is in contrast to very many other reptile groups and the usual summary of plesiosaur bone microstructure (e.g. de Ricqlès 1976; Wiffen et al. 1995). Zonation in plesiosaurs has been reported in Late Cretaceous propodials, ribs (Seitz 1907; Enlow & Brown 1957) and vertebrae (Wiffen et al. 1995; Fostowicz-Frelik & Gazdzicki 2001), while other studies report on no cyclic growth in vertebrae and girdle bone (Wiffen et al. 1995; Salgado et al. 2007) and unclear zonal formation in femur (Gross 1934).

One juvenile metapodium in this study (PMO 220.401) shows a circumferential vascular orientation with very visible rings made of trabeculae (Figs. 10G, H and 12A). This is observed in two other plesiosaur skeletons from Svalbard: the juvenile *species E* SVB 1450 (J. Hurum, pers.comm.) and the juvenile *species F* PMO 220.787 (Fig. 15). It is remarkable that only these three specimens show this feature, while the others have unorganized trabeculae throughout. The “rings” might indicate that there is a difference between these specimens and the others, either adaptive or for reasons due to ontogeny, phylogeny or other factors.

It is tempting to call these features “growth rings”. But what is usually referred to as growth rings, or more correctly, zonation, appears in a compact periosteal cortex as zones, annuli and lines of arrested growth. Such a cortex is commonly avascular, and sometimes vascular, the latter a situation encountered in several dinosaurs (Reid 1996). In the Svalbard specimens the feature appears in a highly vascularized and porous cortex and resembles more the situation encountered in the leatherback turtle (Rhodin 1985), the placodont *Placodus* (de Buffrénil & Mazin 1992) and the ichthyosaur *Omphalosaurus* (de Buffrénil & Mazin 1990) than zonation in crocodiles, most turtles and mammals such as the manatee (Fawcett 1942; Rhodin 1985).

In an adult *Placodus* specimen (de Buffrénil & Mazin 1992) up to eight layers of compact bone without vascular canals, called growth marks (*marques de croissance*) was found in a well vascularized periosteal cortex. It is quite similar to the situation in this study but the rings are less well defined due to the presence of more trabeculae in the areas between the marks. It is also similar to the PMO 220.401 in having very small canals in the outermost row. In a Triassic *Omphalosaurus* from Svalbard (de Buffrénil & Mazin 1990) the outer part of the periosteal cortex has a similar stratified appearance. A studied leatherback turtle (Rhodin 1985, fig. 10) had two “growth rings” in a well vascularized cortex and from a study of the figure, also additional circumferential vascular organization like the previous examples.

In these three studies, cyclic growth has been suggested as the reason for the “rings”, but the reason and the frequency of these cycles are unknown. For all the three species, rapid growth has been interpreted from other microstructural characters such as woven bone. This need not be a contradiction to cyclic growth, which is known from mammals as well as reptiles. Dinosaurs that show growth rings often have fibro-lamellar bone, indicating that they had both cyclic and rapid growth (Chinsamy & Dodson 1995). The cyclic growth might however be less pronounced than in other animals, and not include complete halts in the growth, only periods of slower growth, where the compact lines are made (de Buffrénil &

Mazin 1990, 1992). The frequency is suggested to be annual, but this is not sure (Rhodin 1985; de Buffrénil & Mazin 1992).

The reason for the apparent cyclic growth might be related to the environment that the plesiosaurs encountered in the high-boreal regions around Jurassic Svalbard. Two other ichthyosaurs in the study of *Omphalosaurus* (*Stenopterygius* from the Lias of Holzmaden and *Ichthyosaurus* from the Kimmeridgian of France) do not have any striation and the reason might be the “peculiar conditions in the northern habitat of *Omphalosaurus*” (de Buffrénil & Mazin 1990: p.445). In mammals, annual zonation is known mostly from marine animals that encounter cold conditions and small terrestrial forms from cold regions (e.g. the porpoise *Phocoena phocoena*, de Buffrénil 1982). They typically show closely paced rest lines in avascular bone. In the beluga whale, there are striations in the periosteal of the radius that resemble the situation in the plesiosaurs in this study, and the workers suggested that water temperature might influence the growth of the bone, but that this needs further investigation (Felts & Spurrell 1966). Migration could also be a possible reason for cyclic growth (Rhodin 1985), and it is possible that some of the plesiosaurs migrated in and out of the coldest sea areas, resulting in the “rings” in some species.

Sex might also be a reason for cyclic growth. In the leatherback turtle the growth might slow down due to reproductive effort by females (Rhodin 1985). This is not likely in the case of the Svalbard plesiosaurs, since all the three specimens with “rings” are juveniles. From this, it could seem as if the “rings” were a feature for all juvenile plesiosaurs. There is however another juvenile in this study (PMO 216.839) that does not have “rings”, so this is not the case. For PMO 220.401, PMO 220.787 and SVB1450 adult conspecifics are not yet known, so whether these species had cyclic growth in contrast to the other Svalbard plesiosaurs, or changed their growth pattern during life, cannot be investigated at the moment.

The “rings” are only found in small long bones, and propodials from the same specimens would need to be investigated to reveal the real distribution of this feature. In some animals, growth marks are only visible in smaller bones because they have a lower rate of bone deposition (Sander 2000). The rings will also only be visible in bones that have a periosteal component.

Another possible reason for the difference in presence of cyclic growth might be endothermy, even though cyclicity is not necessarily connected to ectothermy. For the Svalbard ichthyosaurs, the absence of rings in *Stenopterygius* and *Ichthyosaurus* may be

because they are younger than the Triassic *Omphalosaurus* and thus were more advanced towards endothermy (de Buffrénil & Mazin 1990).

Bernard et al. (2010) used a tooth analysis to find out whether plesiosaurs, ichthyosaurs and mosasaurs could regulate their body temperature and the conclusion was that the animals were able to maintain a stable, high body temperature. This has also been suggested by others because plesiosaurs are large predators. For the Svalbard species it would also seem probable because the sea temperature was relatively low (Ditchfield 1997; Hammer et al. 2011). The leatherback turtle, which has an inner structure quite similar to plesiosaurs (Fig. 3A) is unique among marine turtles because it has mechanisms for keeping the body temperature well above sea temperature (James & Mrosovsky 2004).

High vascularization, fibro-lamellar bone, dense Haversian tissue and absence of cyclic marks have been seen as signs of sustained growth, intensive bone-body fluid exchange, high metabolic rates and suggestive of endothermy (see summary in Chinsamy-Turan 2005). Plesiosaurs resemble dinosaurs in having several of these characteristics, quite extensive Haversian remodeling (Enlow 1969) and high vascularization. This could be seen as signs of increased body temperature, but the discussion around dinosaur physiology is not solved, and many of the microstructural characters are contested (see summary in Reid 1996; Starck & Chinsamy 2002).

Some of the bones also seem to show signs of ceased growth, with peripheral rest lines (Figs. 14A, E, G). This might indicate determinate growth because such rings are known to occur in mammals reaching adult size, and it has been found in some dinosaurs (Chinsamy 1990). This need to be investigated further.

Future research

In many ways, this is a typical bone histology study of fossil material: The material is chosen from what was available for thin sectioning, and good comparison groups (e.g. ontogeny or phylogeny) were not possible. This is a problem in many such studies (Sander 2000). But studies like this are needed to give descriptions of bone structures from new taxa, ontogenetic stages, bones and time periods. It should, however, be viewed as a starting point, not an end result.

Many questions can be answered by the use of histology studies on fossil bones, especially if combined with extant material in comparative studies. One example is physiology, another is anatomy, for instance by understanding the vascularization of the

plesiosaur body more thoroughly, how muscles were attached to the limbs, and from this, how plesiosaurs moved and grew. Studies of bone microstructure can also be used to understand how different bones were subject to different tension, compression and shear, for instance to understand how plesiosaurs ate (e.g. as done for dicynodonts in Jasinowski et al. 2010).

The Svalbard material can be used for more in depth studies. With so much material from a unique environment, quantitative studies on description of bone microstructure (de Boef & Larsson 2007) can be performed, with better experiment design concerning sample locations and sample size. The challenge with thin sectioning as a destructive method can be overcome by using other techniques such as coring (Sander 2000), the negative side being that information on gross internal structure cannot be obtained.

Conclusion

From this study it can be concluded that:

- Studies of gross internal structure and microstructure in fossil plesiosaur bones can give valuable information. This study shows that it is important that different bones are studied, exemplified in this work because there is a large variation between bones from the same limb and between bones from contemporary species inhabiting the same environment.
- The inner bone structure of the Svalbard plesiosaurs fits an active marine animal living in cold water. There are several connections between microstructural characters and specific physiology or growth patterns. Many of the features in the present material are often found in animals with rapid growth and high metabolism, including secondary osteons, high vascularization, pits on the outside of the epiphysis, woven and possibly fibro-lamellar bone.
- Plesiosaur inner bone structure is most similar to extant marine reptiles that have elevated body temperature and are active, such as the leatherback turtles; to large marine mammals, also secondary adapted to life in water; and to advanced reptiles, especially dinosaurs.
- To increase the understanding of both plesiosaur and vertebrate bone growth and evolution, and the return of tetrapod groups to water, special inner structures such as the endochondral cones and the lack of medullary cavity are important to map.
- The long bones have two inner endochondral cones because the endochondral ossification proceeded slower than the periosteal. In juvenile bones the endochondral ossification is not completed, and the trabeculae in the cones thus collapse easier during fossilization.
- The juvenile cortex has mostly primary vascular canals and some primary osteons, while the subadult and adult have abundant secondary osteons in the inner cortex. This shows that the bones were well vascularised throughout life, and that remodeling happened while the bones were growing, even though the remodeling was not enough to destroy the cone structure.
- In some of the plesiosaur bones there are “rings” of trabeculae in porous periosteal cortex. This is not what is normally called zonation, but might well reflect cyclic

growth due to either temperature, migration, sex, or a combination of these. It might also reflect different growth patterns in juveniles and adults.

- Whether plesiosaurs had determinate growth or not cannot be concluded on the basis of this material. There is no presence of secondary ossification centers, but some bones have what might be peripheral rest lines.
- Not all plesiosaurs underwent a transition to a very porous bone structure through ontogeny. More research is needed to understand the mechanical properties of plesiosaur limbs, both in terms of porosity and buoyancy caused by the possible presence of lipids.

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Figure 1. Plesiosaur humerus with visible cone

Left humerus from a juvenile *species A* (PMO 216.839) showing the area of the inner cone and the medullary cavity pressed down, as often observed in the field. Here especially visible in the area of the proximal cone. Scale bar = 25 mm. Courtesy of Nille Ragnhild Staubo Munthe-Kaas.



Figure 1

Figure 2. Early illustrations on plesiosaur propodials

Early illustrations of the inner structure in plesiosaur propodials mentioned in the introduction. Not to scale.

A. *Thaumatosauros mosquensis*, right humerus. Cretaceous. Kiprijanoff 1883a, plate 13, figure 2. **B.** Juvenile *Plesiosaurus neocomiensis*, femur. Longitudinal section. The earliest illustration of the inner cones in plesiosaur long bones. Kiprijanoff 1883b, plate 2, figure 5. **C.** *Litkesaurus*, right humerus, proximal half. Longitudinal dorsoventral section. Cretaceous. Kiprijanoff 1883a, plate 20, figure 2. **D.** Plesiosaur, propodial. Jurassic specimen from the Kimmeridge clay. Longitudinal section. The earliest interpretation of the components of the inner structure. Lydekker 1889, figure 46. **E.** Juvenile plesiosaur propodial. Williston 1903, plate 22, figure 2. **F.** Juvenile plesiosaur propodial, the same bone as in E, longitudinal section. Moodie 1908, figure 4. **G.** Subadult plesiosaur propodial. Longitudinal section. Moodie 1908, figure 8. **H.** Adult plesiosaur, humerus. Longitudinal section. Moodie 1908, figure 9. **I.** Plesiosaur propodial. Diagram for longitudinal section based on analyzed material. Moodie 1916, figure 6.

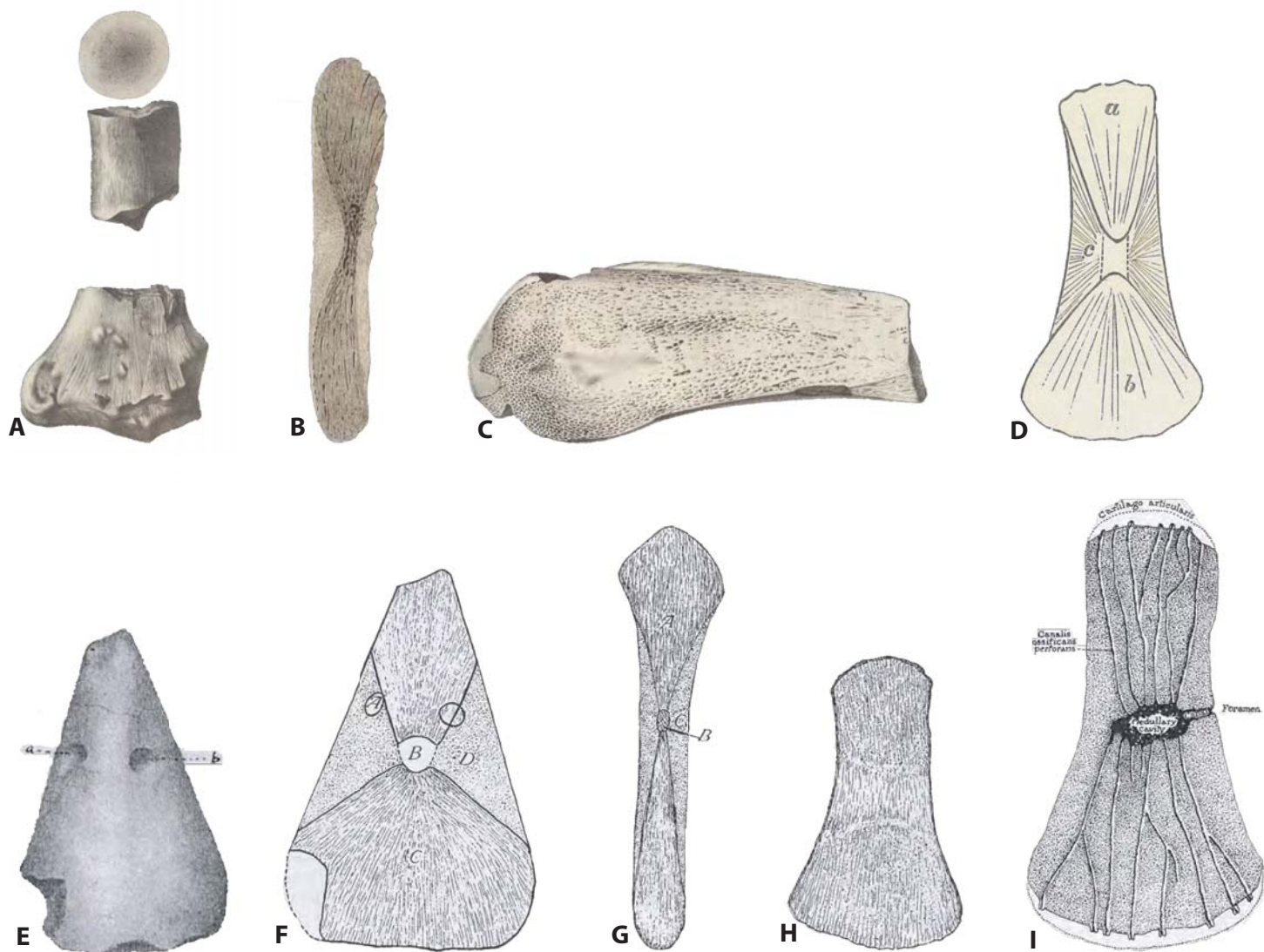


Figure 2

Figure 3. Leatherback turtle (*Dermochelys coriacea*) and mysticete

For comparison to the gross internal structure in plesiosaurs in figure 10.

A. Adult leatherback humerus, inner longitudinal lateral view. **B.** Adult leatherback humerus, transversal view, half. **C.** Adult mysticete (sp.indet.) radius, inner longitudinal lateral view. **D.** Adult mysticete (sp.indet.) radius, transversal view, quarter.

Note the cones in the turtle (A), while the whale (C) has a much more homogenous inner structure, though the periosteal bone is visible. Note also clearly visible growth rings on the turtle (B) compared to the whale (D), which have none. Note also the lack of a real medullary cavity in both specimens. Scale bar = 25 mm.



Figure 3

Figure 4. Map central Spitsbergen

Map for central Spitsbergen and the 2009 locality in Sassenfjorden, where the PMO 216.838 and PMO 216.839 plesiosaurs were collected (marked “2009”). Modified from Hammer et al. 2011.

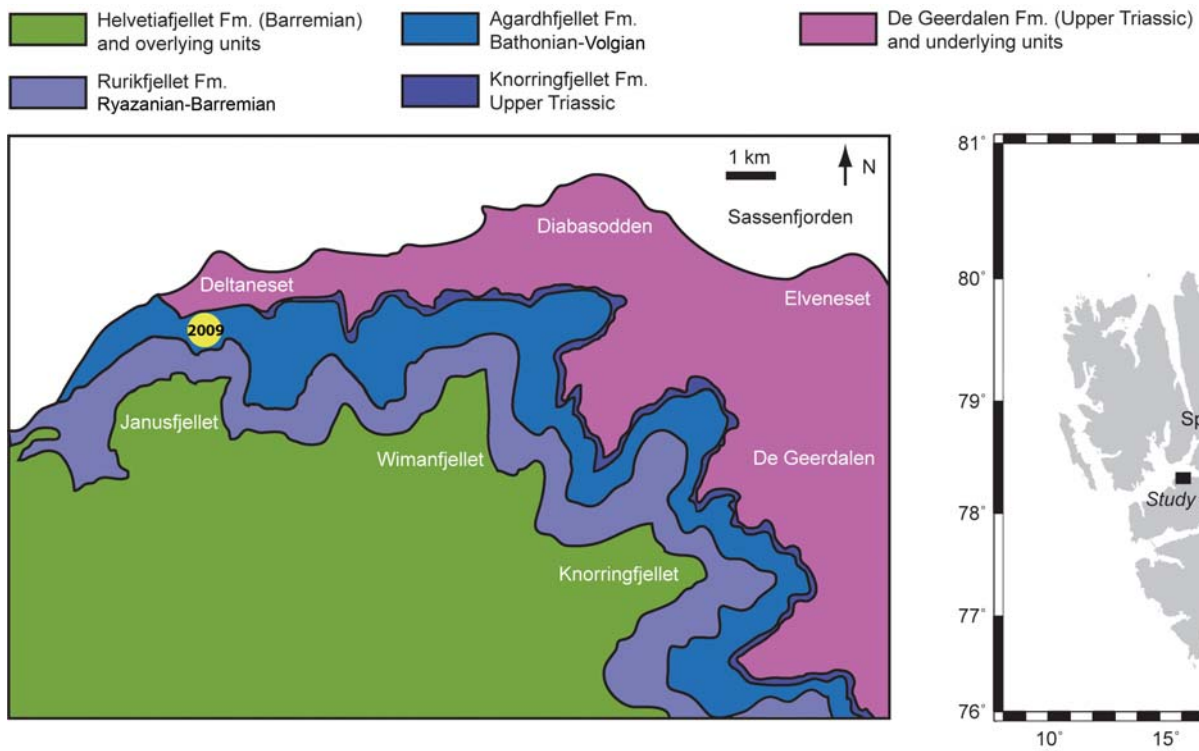


Figure 4

Figure 5. Stratigraphic column

Stratigraphic column for the Slottsmøya member in the Sassenfjorden area. Plesiosaur symbols mark the plesiosaurs used in this study. The position of PMO 220.401 is not verified. Modified from Hammer et al. 2011.

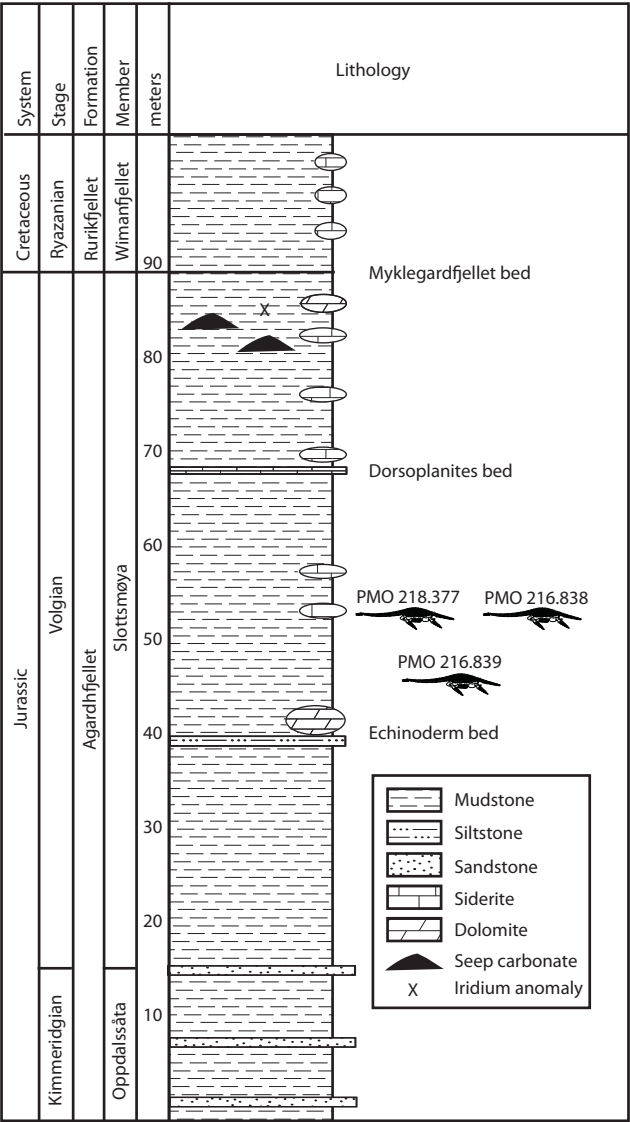


Figure 5

Figure 6. Studied plesiosaur bones

Red = PMO 216.839: humerus, carpal and phalanx. Yellow = PMO 216.838: femur. Blue = PMO 218.377: tarsal and phalanx. Green= PMO 220.401: mesopodium and metapodium (shown on hind limb, but this is not certain). Redrawn from Andrews 1910.

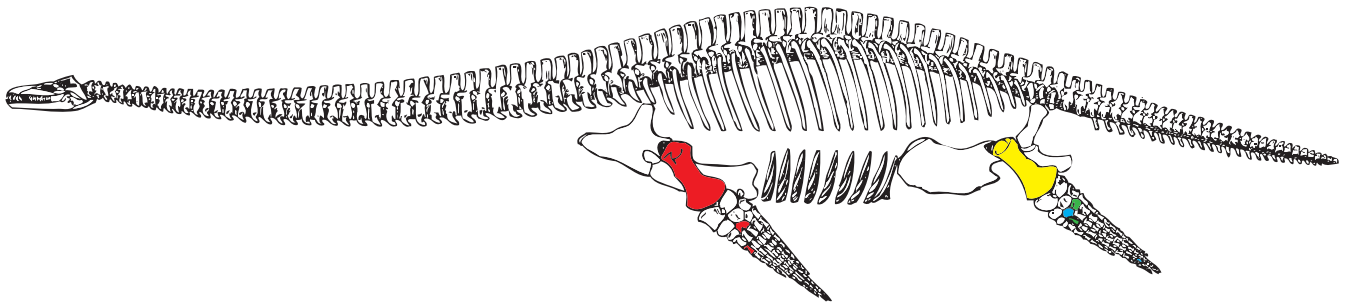


Figure 6

Figure 7. Propodials before sectioning

A. Adult plesiosaur *species B* femur. PMO 216.838. **B.** Juvenile plesiosaur *species A* humerus. PMO 216.839. Scale bar = 25mm



Figure 7

Figure 8. Limb bones

Plesiosaur limb bones thin sectioned in this study. Drawings to the right show positions of thin sections, one transverse and one longitudinal for each bone (Roman numbers and the PMO number together make up the names of the thin sections). All thin sections are dorsoventral, except from PMO 220.401V, which is longitudinal pre-postaxial.

A. Juvenile plesiosaur phalanx. PMO 216.839. Directions unknown, not found articulated. **B.** Juvenile plesiosaur carpal. PMO 216.839. Directions unknown, not found articulated. **C.** Adult plesiosaur phalanx (cast). PMO 218.377. **D.** Adult plesiosaur tarsal. PMO 218.377. **E.** Juvenile plesiosaur metapodium. PMO 220.401. **F.** Juvenile plesiosaur mesopodium. PMO 220.401.

Scale bar= 25 mm. 8D courtesy of Espen Madsen Knutsen.



Figure 8

Figure 9. Propodials, gross internal structure.

Drawings show position of thin sections (Roman numbers and the PMO number together make up the names of the thin sections, for directions see frame to the right in the figure) and interpretation of the photos.

A-C. Adult plesiosaur femur. PMO 216.838. **A.** Longitudinal pre-postaxial cut. Thin section I and VII are from the same spot, but of different thickness. **B.** Dorsoventral cut, half section, dorsal to middle. Distal part mirrored. **C.** Transversal view, quarter of section, outer to middle. Section taken from the proximal part of diaphysis. **D-F.** Juvenile plesiosaur humerus. PMO 216.839. **D.** Longitudinal lateral cut. Thin section VI and VII are from the same spot, but of different thickness. **E.** Dorsoventral cut, half section, dorsal to middle. **F.** Transversal view, quarter of section, dorsal to middle. Endochondral part is missing in the material. Section taken from distal part of diaphysis.

Scale bar= 25 mm. Abbreviations **c**=cortex, **m**=medulla, **n**= nutrient artery, **y** = medullary cavity, **z**= cartilage cap (estimated size).

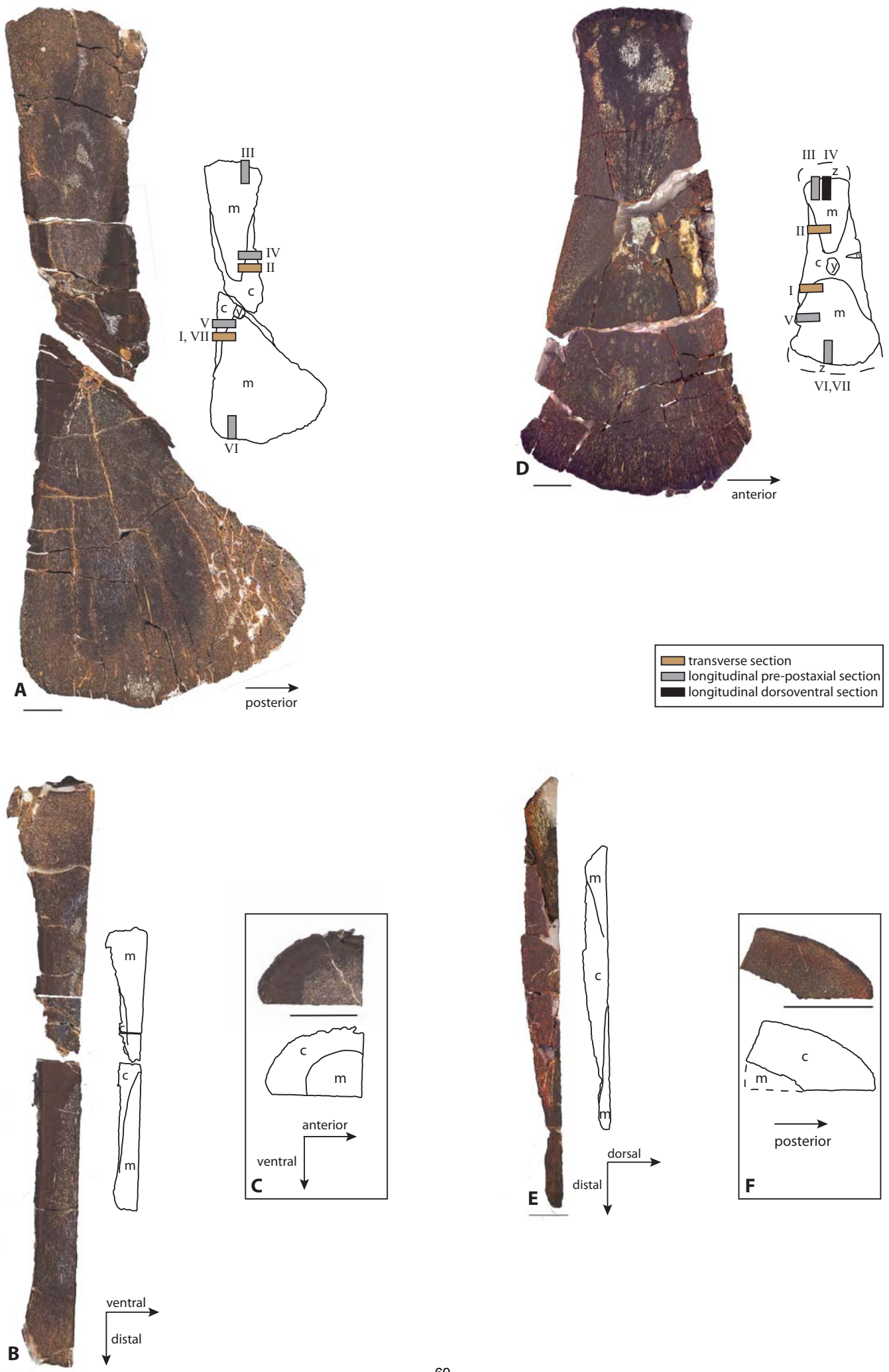


Figure 9

Figure 10. Thin sections from plesiosaur limb bones.

Gross internal structure. All sections except A, C, E and K are sections from the middle of the bone to the outer edge. Longitudinal cut have epiphysis pointing upwards. Roman numbers and the PMO number together make up the names of the thin sections. The pictures are coloured digitally, corresponding to the letter abbreviations for different areas of the bones.

A-D. Juvenile plesiosaur PMO 216.839. **A.** Carpal transversal section (X). **B.** Carpal longitudinal dorsoventral section (XI). **C.** Phalanx transversal section (VIII). **D.** Phalanx longitudinal dorsoventral section (IX). Note the cone-shape in longitudinal section. **E-H.** Juvenile plesiosaur 220.401. **E.** Mesopodium transversal section (III). **F.** Mesopodium longitudinal section (IV). **G.** Metapodium transversal section (I). **H.** Metapodium longitudinal section (II). **I-L.** Adult plesiosaur PMO 218.377. **I.** Tarsal transversal section (III). **J.** Tarsal longitudinal dorsoventral section (IV). **K.** Phalanx transversal section (I). **L.** Phalanx longitudinal dorsoventral section (II), note the large vascular canals in longitudinal direction from the middle to the epiphysis.

Scale bar = 5mm. Abbreviations: **c**=cortex, **m**=medulla, **n**= nutrient artery, **t**= area with collapsed trabeculae.

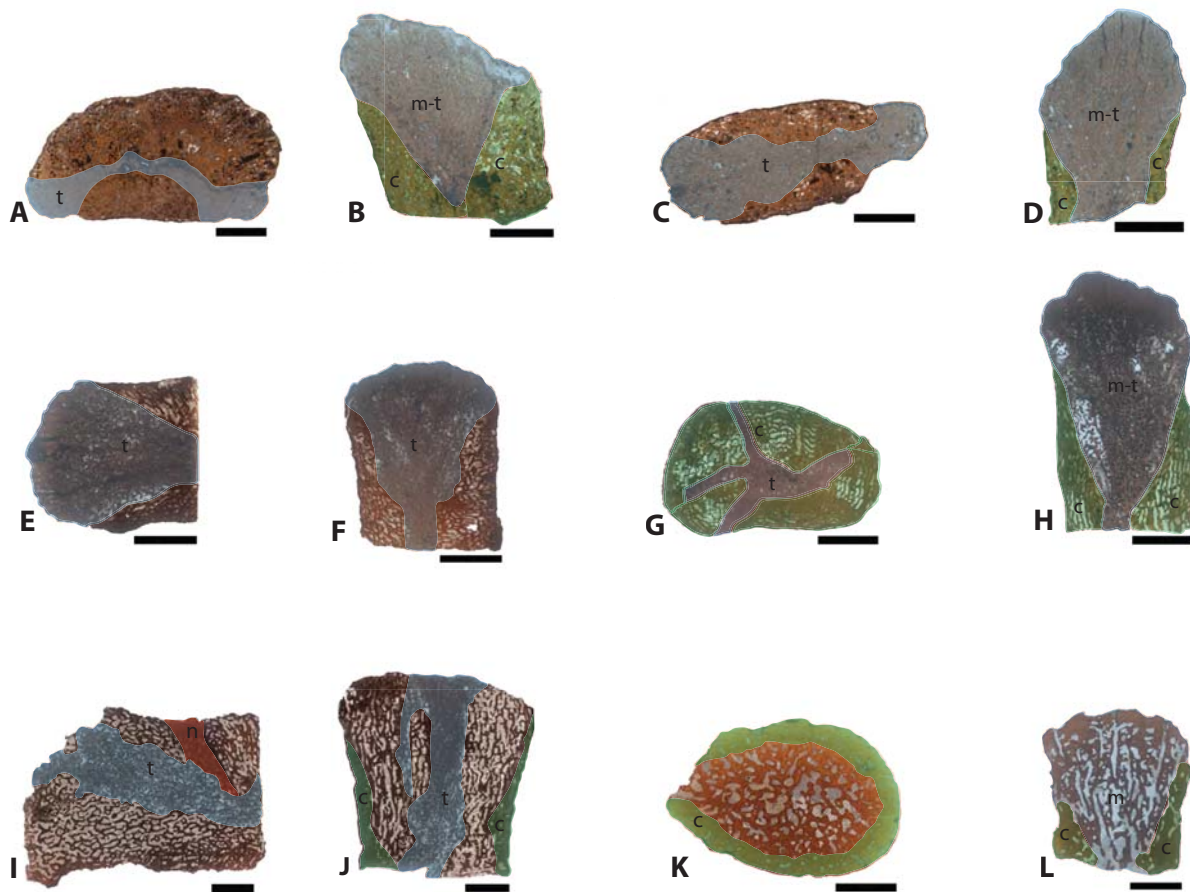


Figure 10

Figure 11. Microstructure PMO 216.839 humerus and carpal.

All pictures are taken with normal light unless otherwise specified.

A. Humerus cortex. Longitudinal canals, mostly simple vascular. Section 216839II, 5x. **B.** Same as A, but with polarized light and gypsum filter. Note woven bone. **C.** Carpal cancellous cortex of complete trabeculae to the right, collapsed to the left. Section 216839X, 5x. **D.** Same as C, but with polarized light and gypsum filter. Section 216839X, 5x. **E.** Humerus medulla. Note decomposition on the right side of picture and the large canal leading towards epiphysis at the left. Section 216839VI, 5x. **F.** Humerus cortex in dorsoventral cut showing longitudinal canals. Section 216839V, 2,5x. **G.** Same as F, but with polarized light and gypsum filter. Section 216839V, 2,5x.

Abbreviations: **c** = cortex, **d** = decomposition, **po** = primary osteon, **t** = area with collapsed trabeculae, **v** = simple vascular canals, **w** = woven bone.

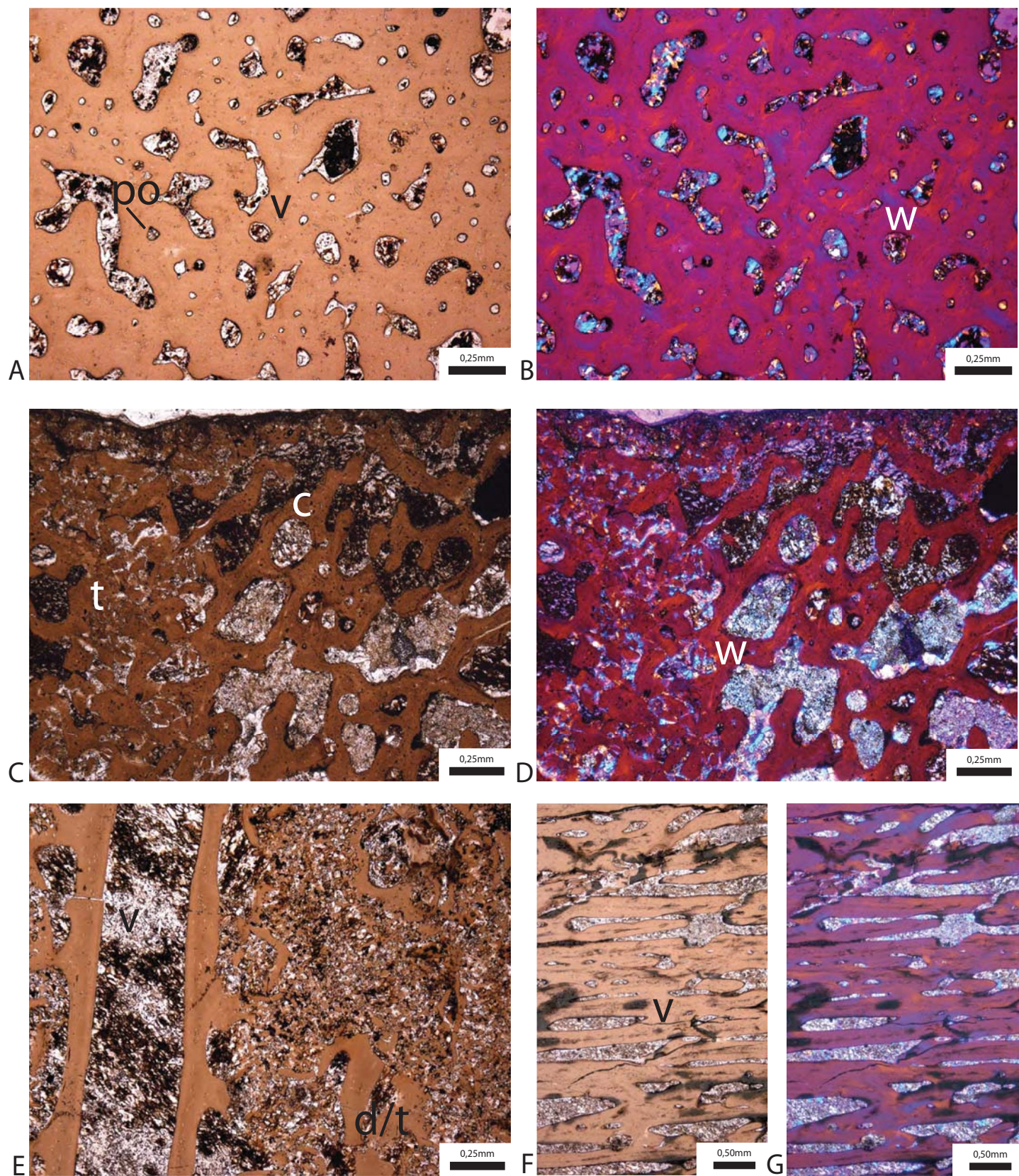


Figure 11

Figure 12. Microstructure PMO 220.401 metapodium, PMO 220.401 mesopodium and PMO 218.377 tarsal.

All pictures are taken with normal light unless otherwise specified.

A. Metapodium cortex. Note the trabecular rings, and that there are smaller cavities furthest out, the large canal and brecciated trabeculae to the left. Section 220401I, 2,5x. **B.** Mesopodium medulla. Epiphysis with collapsed trabeculae and clay mineral infilling. Section 220401III, 2,5x. **C.** Mesopodium. One area with especially high density of osteocyte lacunae. Section 220401III, 40x. **D.** Mesopodium, pre-postaxial section. Note small vessels in inner area, higher porosity further out and degradation. Section 220401V, 2,5x. **E.** Tarsal. Bone wall of nutrient artery. Section 218.377III, 2,5x. **F.** Same as E, but with higher magnification. Note remodeling over lamellar bone. Section 218.377III, 10x.

Abbreviations: **c** = cortex, **d** = decomposition, **l** = lamellar bone, **n** = nutrient artery, **o** = osteocyte lacunae, **po** = primary osteon, **so** = secondary osteon, **t** = area with collapsed trabeculae, **v** = simple vascular canals.

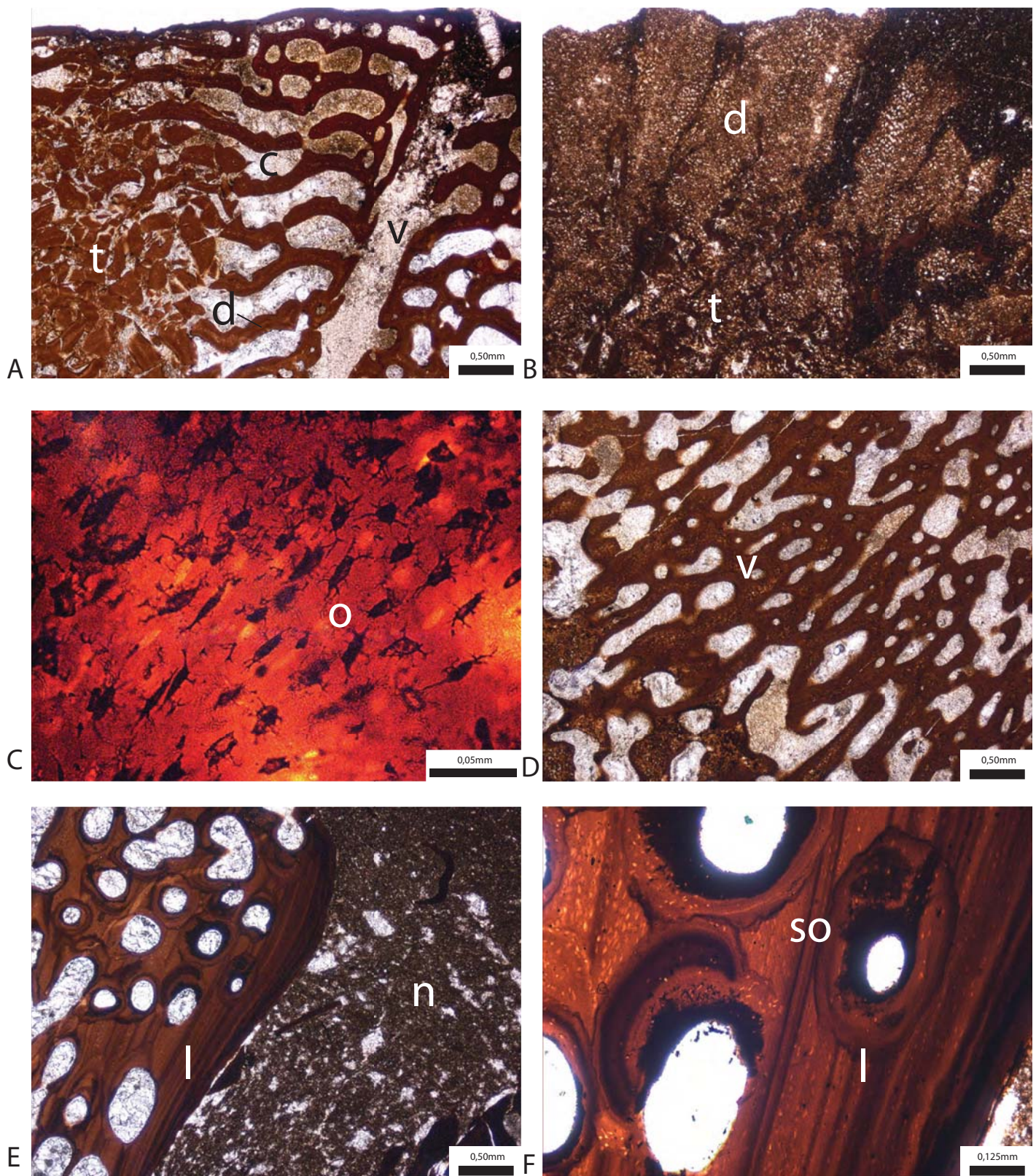


Figure 12

Figure 13. Microstructure PMO 216.838 femur.

All pictures are taken with normal light unless otherwise specified.

A. Cortex. c1 and c2 shows the two cortex types, c1 more compact and with resorption cavities and c2 further in with osteons. Section 216.838I, 2,5x. **B.** Transition between cortex and medulla. Note the remodeling that has been happening in the medulla and the high density of secondary osteons in the innermost part of the cortex. 216.838II, 2,5x. **C.** Same as B, but with higher magnification. Note numerous osteocytes in medullar trabeculae. Section 216838VII, 5x. **D.** Same as C, but with polarized light and gypsum filter. Note decomposition in the middle of several trabeculae. Section 216838VII, 5x. **E.** Medulla, distal epiphysis. Note the decomposition. Section 216838VI, 5x. **F.** Medulla, proximal epiphysis. Section 216838III, 2,5x. **G.** Transition between cortex and medulla, longitudinal dorsoventral cut. Note compact cortex and the vascular system. Section 216838IV, 2,5x.

Abbreviations: **c** = cortex, **d** = decomposition, **m** = medulla, **o** = osteocyte lacunae, **po** = primary osteon, **r** = resorption cavity, **so** = secondary osteon, **vs** = vascular system (unspecified), **w** = woven bone.

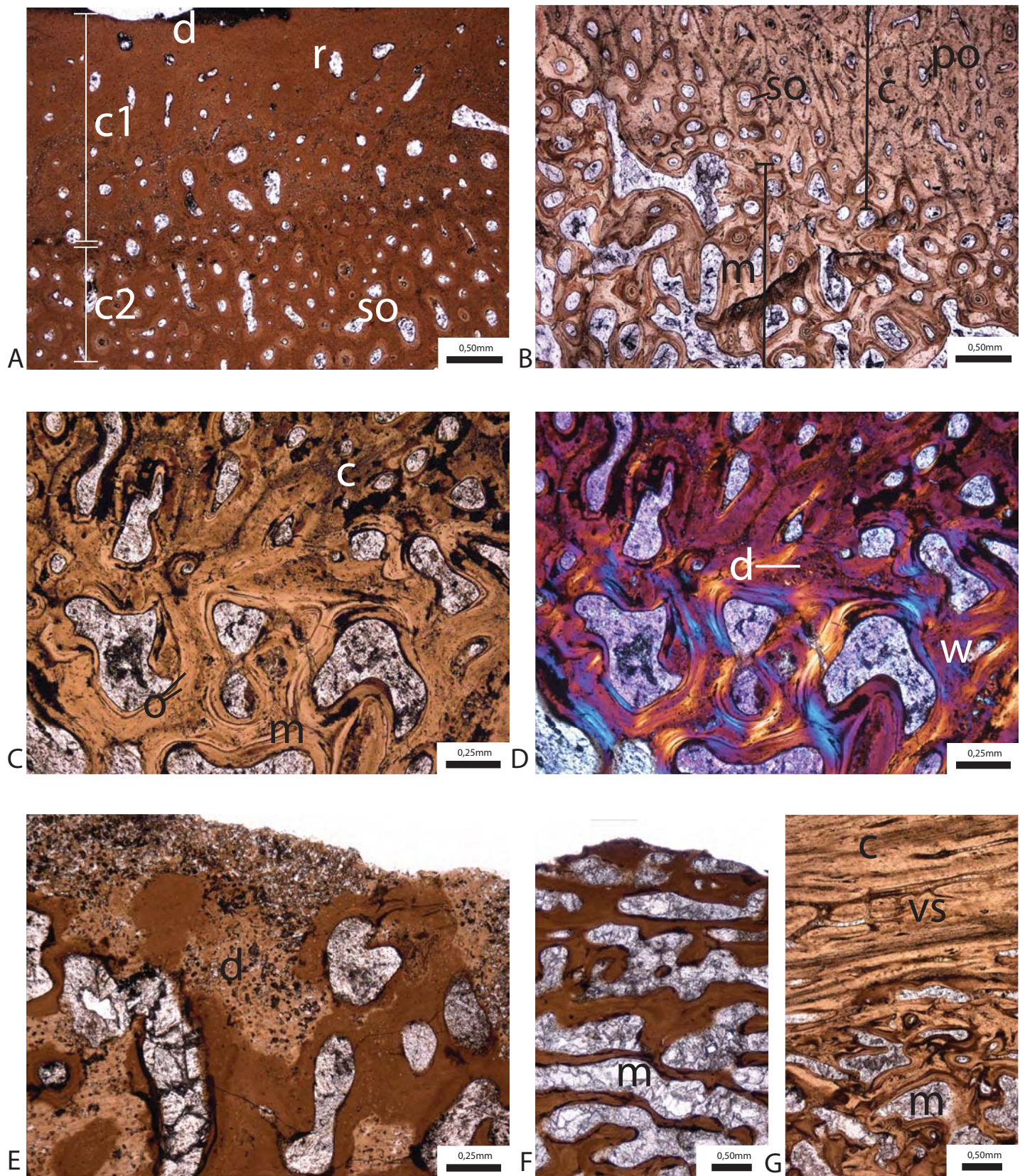


Figure 13

Figure 14. Microstructure PMO 218.377 phalanx.

All pictures are taken with normal light unless otherwise specified.

A. Cortex and medulla. Note remodeling in medulla and possible peripheral rest lines. Section 218.377I, 2,5x. **B.** Same as A, but with polarized light. Note that the peripheral rest lines almost disappear. Section 218.377I, 2,5x. **C.** Medulla. Note the secondary osteons. Section 218.277I, 10x. **D.** Same as C, but with polarized light. Section 218.377I, 10x. **E.** Cortex in longitudinal cut. Section 218.377II, 2,5x. **F.** Same as E, but with polarized light. Section 218.377II, 2,5x. **G.** Cortex with possible rest lines, higher magnification. Section 218.377I, 10x. **H.** Same as G, but with polarized light. Note that the lines almost disappear. Section 218.377I, 10x. **I.** Same as G, but with polarized light and gypsum filter. The lines are slightly visible. Section 218.377I, 10x.

Abbreviations: **c** = cortex, **d** = decomposition, **m** = medulla, **p** = peripheral rest lines, **so** = secondary osteon.

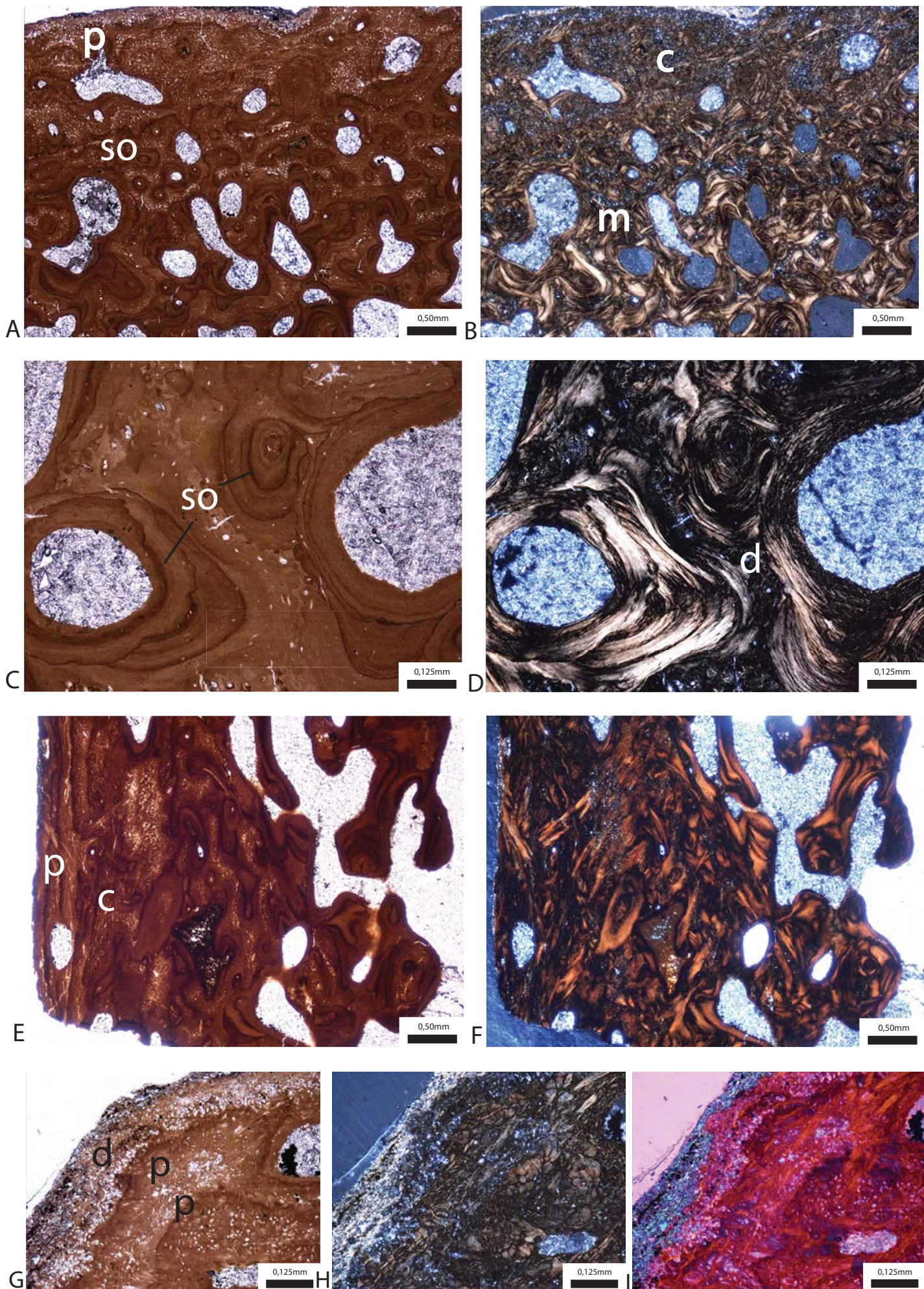


Figure 14

Figure 15. Phalanges showing circumferential vascular orientation.

Phalanges from PMO 220.787.

A. Larger phalanx showing weak “rings”. **B.** Smaller phalanx with clearly defined “rings” of trabeculae and vascular canals. Scale bar = 5mm. Courtesy of Espen Madsen Knutsen.



Figure 15

PMO	Ontogeny	Fig.	H	F	Ms	Mt	C	T	P	Thin section	Comment
216.839	juvenile	7B	X							216.839I-VII	
		8B					X			216.839X,XI	#35 on field drawing
		8A							X	216.839VIII,IX	#9 on field drawing
220.401	juvenile	8F			X					220.401III-V	
		8E				X				220.401I,II	
216.838	subadult	7A		X						216.838I-VII	
218.377	adult	8C						X		218.377III,IV	
		8D							X	218.377I,II	

Table 1. Studied plesiosaur bones

Figures mentioned here are for the original bones before thin sectioning. Abbreviations: **C**= carpal, **F**= femur, **H** = humerus, **Ms**= mesopodium, **Mt**= metapodium, **P**= phalanx, **T**= tarsal.

Errata

- p. 33, 1st paragraph, 2nd line: delete juvenile and add it instead in 1st line, 2nd sentence after "In a "
- p. 44, last reference on page: capitalized letters first in the words of the title should have been small
- same: Farnum 2007 reference p 46
- same: Thewissen and Taylor 2007 reference p 50
- p 56, 2nd sentence, should refer to figure 9, not 10
- p 56: "lateral" should be replaced by "pre-postaxial"
- p. 68, 2nd paragraph after "A-C", "Adult" should be replaced by "Subadult"
- p. 68: "lateral" should be replaced by "pre-postaxial"